International Society for Microbiology

SECOND INTERNATIONAL CONGRESS FOR MICROBIOLOGY

LONDON, 25 JULY—1 AUGUST, 1936

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REPORT OF PROCEEDINGS

EDITED FOR THE EXECUTIVE COMMITTEE

BY

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* Sir Arnold Theiler died on the eve of the Congress and Prof. E. O. Jordan and Prof. F. Paschen are since deceased.

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PREFACE

IN presenting to subscribers and others the following record of the proceedings of the Second International Congress for Microbiology, London, 1936, the Executive Committee, over which I have the honour to preside, desires, at the outset, to express its great indebtedness to Dr. R. St. John-Brooks, its Honorary Secretary, for undertaking the onerous task of editing the varied material that seemed to merit inclusion.

With regard to the papers communicated at the Congress, the Executive Committee decided, almost at its inception, that only brief abstracts would be accepted for publication at the time of the Congress and that but slight amplifications of these would be permissible in the Final Proceedings. To this resolution the sectional committees have adhered, so far as humanly possible, in the firm belief that publication in full of scientific communications to international congresses is in these days no longer called for. Nevertheless, the reader of this record cannot fail to realize the wide interest which the prescribed discussions in all sections evoked and which is reflected in the mass and variety of the scientific matter set forth.

The London Congress owed what measure of success it attained as a scientific and social gathering, not only to the labours of its Executive Committee, but also to the many helpers and well-wishers enrolled in its ancillary committees, whose services proved invaluable in the later stages of its preparation. To all I offer my grateful thanks. An attendance of well over a thousand members from all parts of the world was as gratifying as it was unexpected, and for this widespread international support the Executive is deeply indebted. The Committee is pleased to think that the London Congress may have made some material contribution towards the strengthening of an already world-wide bond of amity and co-operation in scientific effort.

The constitution of the new International Association of Microbiologists, adumbrated on the closing day of the London Congress and under which we shall meet in future, will, we are confident, still further strengthen that bond and ensure the continuity of our triennial gatherings for the mutual interchange of thought, which only personal contact can truly enlighten.

J. C. G. LEDINGHAM

LISTER INSTITUTE,
LONDON,
January, 1937

SECOND INTERNATIONAL CONGRESS FOR MICROBIOLOGY

London, July 25—August 1, 1936

INCEPTION OF THE CONGRESS

At the conclusion of the First International Congress for Microbiology, held in Paris in July, 1930, an invitation from the German National Committee of the International Society for Microbiology to hold the Second International Congress in Berlin in 1933 was accepted in plenary session with acclamation. Owing, however, to the international economic situation which subsequently developed, the proposed Berlin Congress was postponed until 1934 and on further consideration it was found impracticable to hold the next congress in Germany in any circumstances.

In order to surmount this difficulty and to secure the continuity of congresses, the National Committee for Great Britain and Ireland, at the request of the President of the Society—Professor Jules Bordet—agreed in principle to organize a congress to take place in London in 1936. This proposal was favourably received by the other National Committees of the International Society and at a meeting of the British National Committee, held on October 23, 1934, Professor J. C. G. Ledingham, Director of the Lister Institute of Preventive Medicine and President of the National Committee since its foundation, was invited to act as President of the forthcoming Congress and as Chairman of the Executive Committee, which was constituted as follows:

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Note: The late Capt. S. R. Douglas was also a member of the Executive Committee.

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The Executive Committee, which held its first meeting on November 2, 1934, drew up a provisional programme defining the scope of the scientific business of the Congress. The work of the Congress was divided into eight sections, the organization of which was entrusted to sectional conveners, who were members of the Executive Committee with special knowledge of the various subjects. Each Section was empowered to appoint its own executive officers and to draw up a programme for subsequent ratification by the Executive Committee. The final arrangement and the personnel of the Sections was as follows :

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LONDON, JULY 25—AUGUST 1, 1936

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Dr. G. F. PETRIE, Lister Institute, Elstree, Herts, England.

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The following ancillary committees were appointed to assist in the organization of the general management of the Congress and of its social and scientific functions :

LADIES' COMMITTEE

Lady CHRISTOPHERS (*Chairman*), Miss CHICK, Lady DALE, Mrs. DRUMMOND, Mrs. DUNCAN, MRS. HAMILTON FAIRLEY, Mrs. FLEMING, Mrs. HENDERSON, Mrs. LAMPITT, Mrs. LEDINGHAM, Miss LEDINGHAM, Mrs. MINETT, Mrs. RAISTRICK, Miss ROBERTSON, Mrs. ROBISON, Mrs. ST.JOHN-BROOKS, Mrs. SCOTT, Mrs. HENDERSON SMITH, Mrs. VAN NOORDEN, Miss D. B. STEABBEN, Lister Institute of Preventive Medicine, London, S.W.1 (*Secretary*).

FINANCE COMMITTEE

Dr. L. H. LAMPITT (*Chairman*), The Rt. Hon. LORD MOYNE, Dr. F. H. CARR, Mr. BEN DAVIES, Mr. F. W. GAMBLE, Dr. W. D. KENT-JONES, MR. G. E. PEARSON, Major H. LOCKWOOD STEVENS, Dr. H. M. WALKER, Dr. J. T. DUNCAN (*Secretary*).

MANAGEMENT COMMITTEE

Dr. L. H. LAMPITT (*Chairman*), Mr. M. BOGOD, Dr. E. A. CARMICHAEL, Prof. J. C. DRUMMOND, Dr. J. T. DUNCAN, Dr. F. H. K. GREEN, Prof. J. C. G. LEDINGHAM, Prof. H. RAISTRICK, Mr. J. RAMSBOTTOM, Miss M. ROBERTSON, Dr. R. ST.JOHN-BROOKS, Dr. G. HARDY EAGLES (*Secretary*).

FUNCTIONS COMMITTEE

Dr. L. H. LAMPITT (*Chairman*), Prof. A. C. CHIBNALL, Dr. P. FILDES.

REGISTRATION AND RECEPTION COMMITTEE

Dr. F. H. K. GREEN (*Chairman*), Prof. J. C. DRUMMOND, Col. D. H. GILL, Dr. E. B. HUGHES, Dr. J. H. BUSHILL (*Secretary*).

TRANSPORT AND EXCURSIONS COMMITTEE.

Mr. M. BOGOD (*Chairman*), Dr. P. W. CLUTTERBUCK, Mr. PETER NORMAN.

ACCOMMODATION (MEETINGS) COMMITTEE

Prof. J. C. DRUMMOND, Prof. H. RAISTRICK.

EXHIBITIONS COMMITTEE

Prof. J. C. DRUMMOND (*Chairman*), Dr. J. T. DUNCAN, Dr. A. S. MCFARLANE, Dr. W. J. ELFORD, Major H. LOCKWOOD STEVENS, Dr. J. O. W. BLAND, Dr. C. RUSSELL AMIES (*Secretary*).

SECOND INTERNATIONAL CONGRESS FOR MICROBIOLOGY

GENERAL ARRANGEMENTS

One thousand, one hundred and sixty-seven persons representing sixty-four countries became members of the Congress, which was attended by upwards of 1,000 members. Members were provided with identification badges bearing the effigy of Antony van Leeuwenhoek, the Father of Microbiology. A large number were accompanied by members of their families, for whom special badges were provided.

By kind permission of the College authorities, the Congress was held at University College, Gower Street, London, W.C., where the Sections held their meetings, with the exception of Section 7, for which provision was made at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C., by the courtesy of the School authorities. The Refectories of University College Medical School and of the London School of Hygiene and Tropical Medicine were made available for the use of members during the Congress through the kindness of the responsible authorities.

The Committee of the Cowdray Club, 20 Cavendish Square, London, W., kindly extended the privilege of temporary membership to ladies wearing the Congress badges for members or guests. The Executive Committee wish to record their grateful thanks for private and personal hospitality to members of the Congress and their families provided by various members of Committees and other friends of the Congress, both during the Congress week and subsequently.

THE CONGRESS BADGE

The effigy of Antony van Leeuwenhoek on the Congress badge was modelled from the mezzotint done in 1686 by the early master, Jan Verkolje (1650-1693).

This is generally regarded as the most faithful portrait extant of the Father of Microbiology. The fine impression employed for the purpose of the badge is from the private collection of Mr. George H. Gabb, to whom the Executive Committee is indebted for its use.

The reverse of the medal bears the following legend :

SECOND
INTERNATIONAL
CONGRESS FOR
MICROBIOLOGY
LONDON
1936

GENERAL PROGRAMME

SATURDAY, *July* 25

4.0 p.m. Informal Meeting of Nomenclature Committee.

9.0 p.m. Official Opening of Congress.

SUNDAY, *July* 26

General Excursions.

MONDAY, *July* 27

9.30 a.m. Sectional Meetings.

Scientific Visits and General Excursions.

2.30 p.m. Sectional Meetings.

4.30 p.m. Meeting of Nomenclature Committee.

10.0 p.m. Reception by His Majesty's Government at Lancaster House.

TUESDAY, *July* 28

9.30 a.m. Sectional Meetings.

Scientific Visits and General Excursions.

2.30 p.m. Meeting of Central International Committee and Permanent Commission of International Society for Microbiology.

Sectional Meetings.

4.0 p.m. Meeting of Nomenclature Committee.

8.30 p.m. Reception by the Royal Society of Medicine at the Society's House.

WEDNESDAY, *July* 29

9.30 a.m. Sectional Meetings.

Scientific Visits and General Excursions.

2.30 p.m. Sectional Meetings.

8.30 p.m. Reception by the Royal Society of London at Burlington House.

THURSDAY, *July* 30

9.30 a.m.	Sectional Meetings.
	Scientific Visits and General Excursions.
2.30 p.m.	Sectional Meetings.
	Garden Party at Stoke D'Abernon given by Viscount and Viscountess D'Abernon.
5.0 p.m.	Cinematograph Demonstrations.

FRIDAY, *July* 31

9.30 a.m.	Sectional Meetings.
	Scientific Visits and General Excursions.
2.30 p.m.	Cinematograph Demonstrations.
8.0 p.m.	Official Banquet at the Trocadero Restaurant.

SATURDAY, *August* 1

10.0 a.m.	Closing Plenary Meeting of Congress.
	Scientific Visits, and Excursion to Oxford University.

SUNDAY, *August* 2

Excursion to Cambridge University.

OFFICIAL OPENING OF THE CONGRESS

The official opening of the Congress took place in the Great Hall, University College, London, on Saturday, July 25, at 9 p.m.

The President announced amid acclamation that the following message of welcome had been received from the Patron of the Congress, His Royal Highness the Duke of Kent:

> "It is with much regret that I am unable to be present at your Reception to-night. But, as Patron of the Second International Congress for Microbiology, I should like to take this opportunity of wishing you all success in your deliberations."

The Opening Ceremony was performed by the Rt. Hon. J. Ramsay MacDonald, Lord President of the Council, who received the Officers and the Official Government Delegates to the Congress. The names and countries of the Delegates are given on page 559 of these Proceedings. Professor Thorvald Madsen, a Vice-President of the Congress, thanked Mr. MacDonald for his presence and spoke appreciatively of the support given by His Majesty's Government to the furtherance of scientific work in many microbiological fields, particular mention being made of the activities of the Medical Research Council, the Department of Scientific and Industrial Research and the Agricultural Research Council, working under the ægis of His Majesty's Privy Council.

On the motion of the President the Congress agreed with acclamation to send the following telegram to His Majesty the King:

> "Members of the Second International Congress for Microbiology, now meeting in London, beg leave to offer respectful greetings to His Most Gracious Majesty King Edward VIII."

Later in the evening the President announced that the following message had been received from the Private Secretary to His Majesty the King:

> "The King greatly appreciates the message addressed to him by the members of the Second International Congress for Microbiology, which opens in London to-day. His Majesty takes this opportunity of wishing the delegates all success in their deliberations."

ADDRESSES BY THE PRESIDENTS OF HONOUR

Professor JULES BORDET (Brussels) :

En ma qualité d'ancien Président, je crois devoir exprimer à nos Collègues anglais toute notre reconnaissance. C'est grâce à eux, en effet, que l'institution des Congrès de Microbiologie a pu se perpétuer. Une période critique, signalée par des incidents imprévus sur lesquels il est inutile d'insister, a dû être traversée. Mais M. Ledingham et ses collaborateurs ont surmonté avec beaucoup de zèle toutes les difficultés, et l'on est en droit d'espérer maintenant que les Congrès se suivront désormais à des intervalles réguliers et viendront consolider, entre les microbiologistes des diverses nations, les liens de collaboration et de cordialité.

Lorsque j'eus l'honneur, il y a six ans, d'ouvrir le Congrès de Paris, je tentai de retracer en quelques mots les progrès réalisés par notre science au cours des dernières années. Depuis notre réunion de 1930, la marche de l'investigation ne s'est pas ralentie. L'étude de la spécificité et particulièrement des antigènes artificiels dits chimiospécifiques s'est poursuivie de façon très brillante, et l'on a recueilli de nouveaux exemples attestant que les anticorps peuvent distinguer des antigènes qui ne diffèrent que par des particularités très délicates de stéréoisomérie. D'autre part, on discute encore la question de savoir si la réaction de l'anticorps sur l'antigène obéit aux lois de la chimie classique ou si elle est régie par ces affinités secondaires, plus subtiles et plus énigmatiques, qui président aux phénomènes d'adsorption. Quelle que soit l'intérêt de ces recherches, on ne doit pas perdre de vue que, dans le domaine de la spécificité, le problème le plus important à résoudre est d'ordre physiologique plutôt que d'ordre chimique. La question essentielle est, en effet, de savoir comment l'organisme parvient à élaborer, quel que soit l'antigène administré, un anticorps toujours spécifiquement approprié.

On a consacré aux recherches concernant les virus invisibles l'inlassable persévérance que le sujet exige. Il n'est point de problème plus passionnant, puisqu'il comporte l'étude de la vie dans ces mystérieuses profondeurs où les dimensions deviennent tellement petites que parfois l'on se demande si l'on est encore en présence d'êtres vivants et si les phênomènes qu'on observe ne sont pas dûs à des principes chimiques doués de propriétés imprévues. Et la question la plus troublante qui se pose à ce propos, l'une de celles qui dominent toute la médecine d'aujourd'hui, n'est-elle pas de savoir si des tumeurs malignes, créées par des agents purement chimiques, semblables à ceux qu'on trouve dans le goudron, peuvent se propager et se transmettre par greffe sans qu'à un moment donné, et à l'insu de l'expérimentateur, un virus invisible intervienne ?

De son côté, la prophylaxie des maladies transmissibles continue à se perfectionner, grâce a l'appoint de nouveaux procédés d'immunisation, qu'il s'agisse de maladies tropicales comme la fièvre

jaune ou de maladies endémiques dans les pays tempérés, telles que la diphtérie et la coqueluche. Chacun sait que l'emploi de l'anatoxine diphtérique s'est beaucoup généralisé. Un moment contestée, l'efficacité de la vaccinothérapie coquelucheuse est aujourd'hui très généralement reconnue, pourvu bien entendu qu'on applique le traitement assez tôt et qu'il s'agisse de cas indemnes de graves infections secondaires. En Belgique, depuis plusieurs années déjà, la plupart des enfants atteints de coqueluche sont traités par le vaccin, dont les pédiâtres apprécient les effets bienfaisants.

Avec une sagacité parfaite, les organisateurs du Congrès ont choisi, parmi tant de problèmes, ceux qui méritent le mieux d'être soumis à nos échanges de vues. Le programme qui nous est offert est aussi attrayant que possible, et nous allons avoir le privilège de passer à Londres, dans une atmosphère aussi stimulante que cordiale, quelques journées instructives et charmantes.

Sir JOHN MACFADYEAN (London):

I am glad to have the opportunity to say how proud I am of having been appointed a President of Honour at this great Congress, which will be honoured by the presence of so many distinguished men from all parts of the world.

The first Congress of a similar kind at which I was present as a Member was the International Congress of Hygiene and Demography, which was held 45 years ago, in 1891, in London.

It was devoted to the preventable contagious diseases of man, and a separate section considered the contagious diseases of animals communicable to man. The most important diseases of animals that were discussed at that Congress were rabies and tuberculosis, but papers were read and discussions took place on food poisoning, infectious udder diseases of the cow, transmission of disease through milk, and anthrax.

The 1891 Congress was made memorable by a question asked at the opening General Meeting by the late King Edward VII (then Prince of Wales). The question was "If preventable, why not prevented?" It might be made the motto or watchword of the Society of Microbiology.

The question was obviously intended as an incentive to research in these contagious diseases, and there can be no doubt that it had that effect in this country.

I have naturally been led to think of the great advance in microbiology that has been made regarding the contagious diseases of animals since 1891. The discovery of the ultra-microscopic viruses as the cause of many serious diseases of man and animals was one of the most pregnant.

Thanks to her insular position Great Britain has been exceptionally successful in dealing with contagious diseases of animals by the stamping-out method and the prohibition of the importation of live animals. Since 1891 bovine pleuro-pneumonia, rabies and

glanders have been eradicated, and foot-and-mouth disease has, on many occasions, been stamped out, though permanent freedom from it apparently cannot be assured.

During the period a prolonged attempt was made to eradicate swine fever, but the effort was abandoned because the breeders and owners of pigs were apparently not in favour of its continuance. It has always been recognized, at least in this country, that any attempt to eradicate a contagious disease of the animals of the farm must, in advance, have the support of the owners. The stamping out of rabies was long delayed by the opposition of dog owners to muzzling. A committee appointed to investigate bovine brucellosis advised that the disease should be made a notifiable one, but no action was taken apparently because it was thought that it would not have the approval of breeders.

Of the diseases communicable from animals to man, and still continuing, the most important is bovine tuberculosis. Probably complete eradication of the human type of tuberculosis within any foreseeable time cannot be expected, but the eradication of the bovine type must now be regarded as practicable in view of the wide measure of success that has already attended the efforts that are being made in the United States of America. It must be admitted, however, that it would be very difficult in this country because of the much heavier incidence of the disease in British cattle and their higher value, but success would be a greater triumph than any that has yet been achieved in the same direction. It would confer a boon on agriculture, and there is the still greater incentive that it would prevent illness and death in human beings.

Great credit is due to the Ministry of Agriculture and its present distinguished President for the plan of gradual eradication recently begun, but since it is not compulsory its effect will be slow, and meanwhile the infection of human beings with bovine tubercle bacilli in milk will continue.

The principal obstacles to eradication appear to be expense, and apathy on the part of the public. These difficulties have always arisen in connection with efforts to stamp out contagious diseases of animals that are not rapidly fatal, and in the present case the greatest of them is apathy. The general public are not convinced that there is any serious danger of children becoming infected from the cow if what is called clean fresh milk is obtainable, and the family doctor is sometimes of the same opinion.

An increased effort to bring the facts in this connection home to the public is greatly to be desired.

I am, myself, peacefully minded, and would not wilfully say anything calculated to excite tumult or rebellion, but I wish the people would arise and demand that this continued infection of human beings with a preventable cattle disease must now stop.

Alternatively we may have to say that bovine tuberculosis, like the poor, will always be with us.

Sir ROBERT MUIR (Glasgow):

In the few words which I have to offer I shall speak of microbiology from the standpoint of the teacher of pathological science—one may say, from the academic point of view. It is a trite saying that microbiology has now permeated all medicine and surgery and has modified them in every aspect. A new science has come into being, and the extent and rapidity of its growth have rarely been equalled in any other department of human knowledge. This remarkable growth has raised many new questions; for example, with regard to teaching, the relation of teaching to research and specialization within the field of investigation itself. As a teacher for over forty years I have witnessed much of the change and have endeavoured to play a part in meeting the new situation from the medical point of view. It has been no light task, I need hardly say, to incorporate the contributions of microbiology without overburdening or extending the medical curriculum, and I hardly think that anyone will claim that adjustment has yet been made in due perspective. If one thinks of bacteriology, morphological and biological, of infection, of immunity and especially of serology, of anaphylaxis and its relations, of virus diseases, etc., the difficulty of making a due and proportionate selection in relation to the requirement of physician and surgeon becomes readily apparent. In a sense one cannot have too much of any one subject, but with present limitations as to time the share of any one may readily be too much. A sharp distinction should be made between the requirements of those who are to practise medicine and surgery and those who are to practise bacteriology. For the former one can deal only with fundamental methods and principles, and to recognize what is really of fundamental importance is one of the most difficult of the teacher's tasks. Though I have been frequently impressed by the use which an intelligent man may make of even elementary instruction, one cannot make our ordinary medical students competent bacteriologists. If actual bacteriological work is to be seriously undertaken, special post-graduate instruction is essential—this is now accepted—and the proficiency attained should be recognized by diploma or degree. Further, for anyone who is to deal with the problems of disease a sound knowledge of general pathology and pathological histology is essential. The centre of study should be the disease—neither merely the particular bacterium or virus on the one hand, nor merely the tissue damage and reactions on the other. The region into which the organism passes on invading the body should not be unknown to the bacteriologist, should not be one where it cannot be followed by him. The pathologists can and ought to be satisfactorily conversant with bacteriology, and the converse also holds. This is no extravagant requirement—I know many who satisfy it in full degree.

It is now recognized that *intensive* research is almost incompatible with *systematic* teaching—which is often said to be unfortunate, but, on the other hand, this is a welcome indication of the increasing demands of investigation. The position is being met, and I trust will be more and more fully met, by special lectures by investigators. There is no more valuable method of instruction than this—and the lecturer as giver is also blest.

In true perspective the question of specialization is closely related to that of instruction. It may be true that one can nowadays do research of real value in only one special department, but it is not true that the knowledge of the investigator need be restricted to that department. How much knowledge of cognate subjects is possible for anyone must be a matter of trial but it seems to me that unfortunately the trial is often not made. There seems to be a growing tendency to succumb to the fallacy that research in one department will suffer from acquaintance with other departments—this may mean under-estimating one's powers or excessive modesty, but it is unfortunate. So I would put in a plea for breadth of knowledge and wide outlook on the part of investigators. I am sure that this would lead to enhanced interest in science generally and also aid intercourse and co-operation between workers in different fields.

A survey of the Proceedings of this Congress must awake wonder in everyone and enquiry as to how the present position of microbiology has been reached. If one surveys its history over the past years one sees that sometimes a man of vision has arisen and enabled a new field to be occupied by many workers. Sometimes the introduction of a comparatively simple method has led to a like advance. So will it be in the future. It is perhaps unlikely that in the coming years discoveries will be so closely gathered together as in the last two decades of the last century which I think may be called " The Golden Age "—when one arresting announcement followed hard on the heels of another, but with men and methods, as in the past, onward progress is assured and no one can set limit to what may be attained. And in these days it surely counts for much that the results of your investigations are essentially and purely beneficent. I wish you all success in this beneficent work.

Professor George H. F. Nuttall (Cambridge) :

I highly appreciate being called upon to speak before this assembly as one of the Presidents of Honour. It is a reward coming to an old man who, 58 years ago, when a boy, began dabbling with microbiology after his father, a medical man, gave him his first microscope.

What I have to say may be expressed in a few words, beginning, if I may crave your indulgence, in a reminiscent vein.

The discovery of the malaria parasites by Laveran in 1880, and

that of the tubercle bacillus by Koch in 1882, were the most stirring events of my student days. In 1883, I had the thrilling and unforgettable first experience of staining and demonstrating the tubercle bacillus to myself and my fellow students.

From 1885 onward, however, and for many years after, the greatest stimulus came to me through meeting leading men of science and promising younger men who were carrying on research in microbiology in different parts of the world.

Through working in various laboratories abroad, travel on three continents, and attending congresses such as this, I had the opportunity of meeting most of the leading workers in my chosen field of research and happily some of them in course of time became treasured friends. I mention this for the benefit of younger men among us at this Congress, adding the earnest advice to them to acquire sufficient knowledge of one or two foreign languages so that they may be able to converse in a satisfactory manner with foreigners they may meet either at home or abroad, remembering that our problems are universal and that conversing with a man about his work, whether previously read or not, is often most helpful and certainly the best way to establish cordial relations that may last for life.

There are three outstanding romances in pathology which especially struck my imagination in the past. They relate to the fundamental discoveries which elucidated the ætiology of three important diseases. I had the good fortune to know nearly all the workers who solved the problems concerned: I refer to (1) Piroplasmosis in cattle, identified with the name of Theobald Smith, a disease of great economic importance in many parts of the world, (2) Malaria, associated mainly with the names of Laveran, Ross, and Grassi, (3) Yellow Fever, associated mainly with the names of Reed and Gorgas, a disease the deadly effects of which on man and commerce I had witnessed ten years earlier. There are further romances and also some tragedies to be read into the discoveries relating to the ætiology of plague, trypanosomiasis, relapsing-, trench-, typhus- and dengue-fever, etc., which have likewise been shown to be transmitted by arthropods serving as vectors, but these I can do no more than mention. The study of many parasitic organisms affecting man, animals and plants, and the part played by vectors and reservoirs are receiving ever increasing attention, and work on Medical, Veterinary and Agricultural Zoology is growing apace.

To conclude, confining myself to man, I would stress that by far the most important problem facing us to-day is malaria, because it affects about a third of the world's population and the greater part of the British Empire. Ross's view, at one time strongly held, that mosquito control alone would suffice to eradicate malaria, may hold for selected localities, but in most cases we are compelled to turn to chemotherapy. Quinine, the old sheet-anchor, has been

improved upon in some ways, by the discovery of the synthetic specific drugs plasmoquine and atebrin ; nevertheless a better and cheaper remedy is obviously demanded and research directed to this end should be encouraged and diligently pursued.

I have perforce but touched lightly upon a few of the great number of subjects with which this congress will deal and end by wishing it all success. May the contacts established among you lead to new and lasting friendships and may good fellowship prevail.

PRESIDENTIAL ADDRESS

Professor J. C. G. LEDINGHAM :

The Executive Committee which has organized this Second Congress of a new series has done me the honour of inviting me to assume the duties of Congress President and I am deeply conscious of the responsibilities I have undertaken.

On behalf of my Committee I desire at the outset to extend the heartiest welcome to all of you whether you come from near or far. It is particularly gratifying to us that so large and representative a group of members has made the journey from distant parts of the American continent and from the Far East to take part in our deliberations.

We in this country gladly responded to the appeal of the Permanent Commission of the Society to organize this second congress in London and so help to keep in being an institution so admirably calculated not only to promote a fuller comprehension of the scope and achievements of our science—a minor consideration, perhaps, in these days of rapid intercommunication by the written word—but also and above all else, to bring into personal contact workers on similar problems.

It is well for the moment to link ourselves with the past. At the first congress of this series six years ago, we worshipped at the shrine of Pasteur, the founder of the science with whose problems the bulk of the scientific business of our sections will be concerned. In the course of these six years we have had to mourn the passing of many distinguished pioneers and exponents of our Science and I need mention only David Bruce, Beijerinck, Kitasato, Roux, Calmette, Cantacuzène, Welch, Kolle, Theobald Smith and Charles Nicolle. We have also to mourn the death on the eve of this Congress of Sir Arnold Theiler, one of our Vice-Presidents, and a very distinguished microbiologist in veterinary fields.

Pasteur, Lister and Koch we venerate for their pioneer labours in the cause of a science that has revolutionized medical thought and permeated every sphere of economic biology. Microbiology, however, or the lore of little living things, whether they be plants or animals, has a much wider connotation and we have thought fit to honour at this present congress the Father of Microbiology, Leeuwenhoek, by having his picture engraved on our congress

badges. We do this also because of Leeuwenhoek's intimate association and fellowship throughout his long working life with the Royal Society of London, which next week is to honour us.

This new series of congresses came into being, as you are aware, through the efforts of the Health Section of the League of Nations in 1927 to reconstitute that free and friendly exchange of scientific opinion which a world war had broken. In so far as the scope of its programme is concerned, it represents the direct continuation of those sections of Hygienic Microbiology which for over forty years figured largely in the business of the International Congresses of Hygiene and Demography, of which the fifteenth and last took place in Washington in 1912. The London Congress of 1891 had, not only a distinguished section of Hygienic Microbiology with Sir Joseph Lister as its President and a whole galaxy of renowned pioneers participating in its business, but also an equally important section which dealt with the relation of diseases of animals to those of man. Of the officers of this latter section Sir John McFadyean happily survives to greet you to-night. It is unlikely that these vast congresses of hygiene would have survived, at any rate in their accustomed form, even had the world upheaval not intervened. The extraordinary development of Bacteriology and of Microbiology generally would have undoubtedly rendered necessary their separation from a programme already top-heavy with the multifarious ramifications of hygiene in its more practical and public aspects. Next week's programme, however, caters fully not only for hygiene but also for many another economic activity, in so far as it depends for its very existence on knowledge of microbic action.

It is true to say that Microbiology owes its present imposing growth to the sheer necessity of exploiting to the full the growing knowledge of bacteria and their potentialities in the interest of a rational pathology, and for the most part, the building up of that imposing growth has been the work of men of medical and veterinary training. The last two decades have witnessed, however, a still more intensive attack on the bacterial stronghold by biochemists and biophysicists eager to probe more deeply by aid of their special disciplines, into the fundamentals of bacterial metabolism and the interactions of germs with the fluids and tissues of their hosts. For them, too, we cater here.

Furthermore, the great advances in our knowledge of virus diseases in quite recent years, and of the agents which cause them, have necessitated still another section to deal with studies in this most fascinating, if difficult, field.

It seems already clear that, in future congresses, microbiology must suffer still further expansion and sub-division if the ground is to be adequately covered. What then should be the main function and purpose of congresses such as this ? A striking feature of present day research in our subject as in every other, is the enormous growth in numbers of the professional research worker in contrast with the

more casual and vocational type of a past age. It arises from the elaboration and wide diffusion of those experimental methods and armamentaria without which any advance is impossible and from the ever-increasing importance to microbiology of collateral disciplines such as biochemistry, biophysics and experimental biology. So intense and widespread is the prosecution of research in all these fields that the notification of any new discovery, however small, by the individual or the team is followed almost immediately by its exploitation at the hands of the multitude.

We seem to know now far more clearly what we want to know and in many fields of enquiry, though unfortunately not in all, both workers and methods are only too easily mobilized to undertake a given task, but I would emphasize that there is still room enough, as I imagine there always will be, for the lone worker destined to carve new pathways that permit advance into unexpected fields. Such is the diffusion of knowledge in these modern days that a real danger exists of yielding to the temptation of premature publicity for which little sympathy will be felt. On the other hand, anxiety to secure the earliest possible publicity may be excused, for it usually arises from a very thorough appreciation of the fact that, in default of it, forestalling is only too likely. Even if priority is secured, it may be by a week or a month, the maintenance of the lead is often impossible, except at the cost of severe mental strain and the irony of it is that this feverish eagerness to exploit may not infrequently be quite out of proportion to the value of the knowledge likely to accrue. Actually it may be an index of the scarcity of really inspired workers.

I mention these undoubted defects of present day mass production of new knowledge, for which there would seem to be no easy remedy, in order to emphasize what I consider to be the main function and purpose of the modern congress. That main function is, in my view, to afford an opportunity for experts to compare notes with each other on subjects of mutual interest, not only inside, but also and preferably, outside the congress halls. Only personal contact, otherwise so lacking in these days of strenuous and highly competitive addiction to research, can give the necessary and very desirable corrective to the written word and the unmerited reverence often paid to it.

An international congress such as this is never purely an assembly of experts in one branch or other of the various spheres of scientific activity represented. Nor is it desirable that it should be, for our subject matter makes an appeal to many vitally interested sections of the population outside the ranks of the laboratory and it is most desirable that public opinion should be correctly instructed in the lore and activities of the smallest living things in nature which are yet so potent for good or ill.

I stress this point because no scientific subject has been more incorrectly presented to public opinion through the lay press than

the cult of microbes. Burlesque of the microbe has in fact too often served as the cloak of ignorance.

As you will agree, the scientific business to be discussed next week is likely to tax your energies, both mental and physical, severely, but it is the great hope of the Executive Committee and myself that outside the sectional meeting rooms and in the course of the week's social functions, members will find ample opportunity for informal discussion of their common problems, that many new acquaintances will be formed, and many old ones renewed. Should a prevailing friendliness and informality of atmosphere prove to be the key-note of this London Congress, the Executive Committee and all the many Sub-Committees that have helped towards its organization, will be abundantly repaid for their labours.

ADDRESS BY THE LORD PRESIDENT OF THE COUNCIL

Mr. Ramsay MacDonald, at the close of the President's address accepted the President's invitation to address the assembly.

It was, he said, a very great pleasure for him to welcome the Congress on behalf of His Majesty's Government. The President's address, founded on a background of knowledge and skill, was encouraging alike to the expert and the interested man of leisure. It exposed a very wide field of endeavour, and indicated how much the future life of humanity depends on the success of such men of science as those assembled at the Congress.

As a Member of Parliament for the Scottish Universities, and in his capacity as Lord President of the Council, he was pleased to be reminded of many familiar names among those who had just been presented to him. The scientific programme of the Congress illustrated the vast scope of its scientific interests. Microbiology from the days of its inception by Leeuwenhoek, and since associated with such names as those of Pasteur, Lister and Koch, was indeed international. In its application to nutrition and health, it influenced the raw materials of human prosperity, and was of great importance in industry. Formerly it could be considered to outride the sphere of Government interest, but now was well within it. He envied members the scientific mentality which they could bring to bear on their problems, and envied them also the discovery of "a pure culture" in an atmosphere free from outside interference.

He was particularly grateful to Professor Madsen for the encouraging remarks he had made with regard to the activities of the Medical Research Council and the other research organizations operating under the auspices of the Privy Council, over which he had the honour to preside. That the publications of these bodies were welcomed and appreciated abroad was a most gratifying tribute to this country's efforts in the cause of scientific research.

CONGRATULATORY ADDRESSES

The following congratulatory addresses were received in connection with the Official Opening of the Congress:

China

On behalf of the National Health Administration of the Republic of China and of the Chinese Society for Pathology and Microbiology, we have the honour to extend to you our very hearty congratulations and sincere wishes for the success of the Congress.

Unaccustomed to scientific thought, China has been drawing largely from the researches of European and American workers. It is only in recent years that we are beginning to contribute our meagre share to the vast store of scientific knowledge. It is, therefore, a source of great satisfaction to us to be able to attend this Congress and to meet with such a distinguished international group of investigators in the field of microbiology.

May this fellowship serve as a small example of international amity in the united effort for the peace, health and betterment of all people.

RICHARD H. P. SIA
F. F. TANG
S. M. TAO

Finland

This is the second time that the microbiologists of the world meet together in congress in order to deal with the problems of microbiology. The representatives of the various countries will to-day express their thanks to the British Government and to their British colleagues, whose efforts have made it possible to convene this meeting. In our country—Finland—we are also well aware of the need for such congresses of microbiologists and of the benefits derived from them. The Government of Finland has sent its official representative; and, in addition, many Finnish microbiologists are among those who take part in the meeting. I have been asked by our Government and by my colleagues to convey expression of our joint gratitude to our British hosts. We are animated by the sincere conviction that this congress of microbiologists—the second of its kind—will be as fruitful of results as the first, which was held in Paris.

O. STRENG

Germany

Als Führer der deutschen Gruppe der Kongressteilnehmer habe ich die Ehre, im Namen der Deutschen Reichsregierung und meiner deutschen Fachgenossen für die Einladung zu diesem Kongress den verbindlichsten Dank auszusprechen. Wir sind der Einladung gern gefolgt, um den gemeinsamen Gedankenaustausch mit unseren ausländischen Fachgenossen auf dem grossen Gebiet der Mikrobio-

logie zu pflegen. Wir begrüssen es, dass der Kongress unter der Aegide des Instituts stattfindet, das den Namen Listers trägt, und dass er uns somit Gelegenheit gibt, den Manen dieses grossen Wohltäters der Menschheit zu huldigen.

Etwa 10 Jahre nach Listers grundlegenden ersten Arbeiten begann in Deutschland Robert Koch seine epochalen Studien, welche ihn zum Begründer der wissenschaftlichen Bakteriologie werden liessen. Nachdem der Kongress an den Wirkungsstätten Louis Pasteurs und Joseph Listers getagt hat, möchte ich nunmehr den Wunsch äussern, dass eine der nächsten Tagungen in Deutschland, dem Vaterlande Robert Kochs, stattfinden möge.

Dem jetzigen Kongress wünschen wir erfolgreiche Arbeit.

R. OTTO

SWEDEN

The Swedish Government expresses thanks for the invitation to be represented at the Congress and sends its best wishes for a good result. The Government hopes that the Congress, where so many nations are represented, will bring its members into closer contact with each other, not only as scientists but as human beings, and that the Congress in this way will contribute to a better understanding between the different nations.

J. FORSSMAN

RECEPTIONS AND OFFICIAL BANQUET

Reception at Lancaster House

On behalf of His Majesty's Government members of the Congress and their families were received by the Rt. Hon. Sir Kingsley Wood, M.P., H.M. Minister of Health, at Lancaster House, London, on Monday, July 27, at 10 p.m.

Reception by the Royal Society of Medicine

A reception was given by the Royal Society of Medicine at the Society's House, 1 Wimpole Street, London, on Tuesday, July 28, at 8.30 p.m. The guests were received by Dr. Robert Hutchison, President of the Society. Miss Lotte Reiniger, maker of "The Little Chimney Sweep" and other silhouette cinematograph film gave a technical demonstration of her art and showed some of h films.

Reception by the Royal Society

A reception was given by the Royal Society of London a Burlington House, Piccadilly, London, on Wednesday, July 2 at 8.30 p.m. The guests were received by Sir Albert Seward, th Foreign Secretary of the Royal Society and a Vice-President o the Congress.

Among the many objects of interest on view were original manuscripts and drawings relating to early work on Microbiology by Fellows of the Society; Grew, Hooke, Leeuwenhoek, Martin Lister, Malpighi and Swammerdam.

Garden Party at Stoke D'Abernon

An afternoon Garden Party was given for the Congress by Viscount and Viscountess D'Abernon at the Manor House, Stoke D'Abernon, near Esher, Surrey, on Thursday, July 30.

Official Banquet

The Official Banquet of the Congress was held at the Trocadero Restaurant, Piccadilly Circus, London, at 8 p.m. on Friday, July 31. Over five hundred guests were present.

The toasts of "The King," "The Sovereigns and Heads of States Here Represented," and of "Our Patron, H.R.H. The Duke of Kent," were proposed by the President. The toast of "The International Congress for Microbiology and Leeuwenhoek's Little Animals" was proposed by Professor Hans Zinsser (Boston, U.S.A.) and replies were made by the President, Professor A. J. Kluyver (Delft, Holland), Professor F. Mesnil (Paris), Professor F. Neufeld (Berlin) and Dr. Edward Mellanby (London). The toast of "The Guests" was proposed

by Dr. Muriel Robertson (London) and replies were made by Sir Frederick Gowland Hopkins (Cambridge), Professor J. Forssman (Lund, Sweden), The Lord Horder, Professor R. E. Buchanan (Ames, U.S.A.) and Professor Jules Bordet (Brussels).

CLOSING PLENARY MEETING

The Closing Plenary Meeting of the Congress was held at University College, London, W.C., at 10 a.m., on Saturday, August 1, 1936.

The President, Professor J. C. G. Ledingham, addressed the meeting and announced that an invitation to hold the Third International Congress for Microbiology in the United States of America in 1939 had been received from the American group represented at the Congress. The invitation was accepted with acclamation by the members present.

Resolutions

The following Resolutions, submitted by the Central International Committee and the Permanent Commission of the International Society for Microbiology, were passed by the Plenary Congress:

(1) That the International Society for Microbiology be replaced by " The International Association of Microbiologists."

(2) That the existing National Committees affiliated with the International Society should in future function as national groups associated with the International Association. (There would thus be no enrollment of individuals.)

(3) That each national group should appoint one member only to act on a *Permanent International Commission for the organization of Congresses.* The President of the last Congress would also be, *ex officio,* a member of the Permanent Commission.

(4) That the Chairman of this Permanent International Commission should be the President of the next Congress, elected by his own nationals and his bureau would also be the headquarters of the Commission.

(5) That the Headquarters of the International Association of Microbiologists should also during the interval between Congresses be the local executive bureau of the country which is organizing a Congress. This bureau would also act, as occasion arose, in liaison with outside organizations desiring the co-operation of microbiologists in scientific projects of international moment.

(6) That on the conclusion of a Congress the Chairmanship of the International Association of Microbiologists should pass to the President who is to prepare the next Congress and the new Headquarters of the Association would be the new President's local Executive Bureau.

(7) That the arrangements for the conduct of each Congress should be drawn up by the President of the Permanent International Commission actually in office, with the assistance of his local bureau. Such arrangements should apply to that particular Congress only. They should, however, be submitted beforehand to each member of the Permanent International Commission and might be amended by a majority recommendation.

(8) That the expenses of the Permanent International Commission as also of the International Association, chiefly clerical and postage, should be a charge on the funds of the local Congress organization from the time of election of the new President to his demission in favour of the next.

(9) That the expenses of Congresses should be purely a charge on the local Organizing body, the main revenue being the subscriptions of attending members. The disposal of surplus funds, if any, would be solely an affair of the organizing Committee.

(10) That the new proposals come into force immediately on the conclusion of the London Congress and that a small drafting committee consisting of Dr. R. St.John-Brooks, Dr. T. M. Rivers and Dr. R. Dujarric de la Rivière be appointed to determine what changes the new proposals necessitate in the statutes formulated in 1930 at the Paris Congress. The report of this committee would in due course be submitted to the Permanent International Commission, which, in turn, would present an agreed statement on the new Statutes to the International Association at the next Congress.

The following Resolutions of the Nomenclature Committee of the International Society for Microbiology were passed by the Plenary Congress :

1. On the Proposals of Prof. R. E. Buchanan and Prof. H. J. Conn relative to the status of the Genus *Bacillus* and its type species, *Bacillus subtilis*.
   - (*a*) It was agreed that *Bacillus* Cohn 1873 should be designated as a *genus conservandum*.
   - (*b*) It was agreed that the type species of *Bacillus* should be designated as *Bacillus subtilis* Cohn, 1872, *emendavit* Prazmowski, 1880[1].
   - (*c*) It was agreed that the type (or standard) strain should be the Marburg strain.

[1] For subsequent confirmatory and detailed description of *Bacillus subtilis*, see Brefeld, O., *Bot. Untersuchungen u. Schimmelpilze*, 4, 36, 1881 ; Gottheil, O., *Zbl. Bakt.* Abt. II, 7, 570, 582, 663, 1901 ; Conn, H. J., *J. Infect. Dis.* 46, 341, 1930 ; Conn, H. J. in *Bergey's Manual of Determinative Bacteriology*, 4 ed., 1934, Baltimore.

(*d*) It was agreed that cultures of the type (or standard) strain of *Bacillus subtilis* together with complete description should be maintained at each of the recognized Type Culture Collections.

(*e*) It was agreed that the genus *Bacillus* should be so defined as to exclude bacterial species which do not produce endospores.

(*f*) It was agreed that the term *Bacillus* should be used as a generic name and that it should be differentiated from the terms "bacillus," "bacille" and "Bazillus" used as morphological designations.

2. On the Proposal by Prof. F. Mesnil relative to generic homonyms, it was agreed that

(*a*) Generic homonyms are not permitted in the group *Protista*.

(*b*) It is advisable to avoid homonymy amongst *Protista* on the one hand, plants or animals (*Metazoa*) on the other.

3. On the Proposal by Prof. R. S. Breed relative to non-capitalization of specific names, it was agreed that

Bacteriologists should accept Article 13 of the International Rules of Zoological Nomenclature, as follows:

"While specific substantive names derived from names of persons may be written with a capital initial letter, all other specific names are to be written with a small initial letter."[2]

The President before declaring the sitting closed, made reference to the very gratifying fact that all the subjects prescribed for discussion in the Sections had elicited the widest interest, to which the admirably sustained attendances throughout the week bore ample testimony. He was glad also to be assured that the social functions of the week had helped in no small measure towards the cementing of friendships and the fuller understanding that comes from personal exchange of views. On behalf of the Executive Committee he tendered thanks to the representatives of the nations for their whole-hearted support of the London Congress and wished them a safe journey to their homelands.

A motion by Professor Bordet that the President receive the thanks of the members for his services was carried with acclamation and after a grateful acknowledgment of this vote of thanks the President declared the Congress at an end.

[2] Examples taken from bacteriological literature: *Salmonella Schottmuelleri* or *Salmonella schottmuelleri*, *Bacillus Welchii* or *Bacillus welchii*, *Acetobacter Pasteurianum* or *Acetobacter pasteurianum*, *Corynebacterium ovis*, *Nitrosomonas javanensis*, *Rhizobium japonicum*.

ABSTRACTS OF SECTIONAL PROCEEDINGS

NOTE.—Abstracts presented by authors who were unavoidably prevented from implementing their intention of being present, are marked with an asterisk.

SECTION 1

GENERAL BIOLOGY OF MICRO-ORGANISMS

DISCUSSION *on* SELECTIVE BACTERIOSTASIS. INHIBITORY ACTION ON THE GROWTH OF BACTERIA AND FUNGI OF (1) SUBSTANCES OF KNOWN CONSTITUTION AND (2) PRODUCTS OF THE GROWTH OF MICRO-ORGANISMS. IMPORTANCE IN THE PREPARATION OF SELECTIVE CULTURE MEDIA

Chairman : Prof. E. Gotschlich (Ankara).

Prof. A. Fleming (London), **Opener** : *Selective Bacteriostasis.*

If we neglect the action of free oxygen in inhibiting the growth of anaerobic bacteria, we are left with a great variety of selective bacteriostatic agents. These may be—

(*a*) Physiological : bile, serum, proteolytic ferments, lysozyme.

(*b*) Products of microbiological activity : staling substances, penicillin, and unidentified products of bacterial growth.

(*c*) Chemicals of known constitution. Almost every chemical shows some selective bacteriostatic action, but it is only necessary to consider those which are outstanding. Eisenberg has worked out the bacteriostatic action of a great variety of chemicals on different bacteria, and Churchman and others have shown the extraordinary selectivity of some of the aniline dyes. Much of the work was dictated by practical ends, as is shown by the great variety of chemicals which have been used in media for the isolation of *B. typhosum*.

Special attention is directed to one physiological bacteriostatic agent, one of microbic origin, and one definite chemical. These are lysozyme, penicillin, and potassium tellurite.

Lysozyme is a ferment contained in most tissues and secretions. (The most potent human secretion is tears and the most potent tissue is cartilage.) Human tears have a lytic action on certain micrococci even when diluted 2,000,000 times, while other bacteria can grow in undiluted tears. This is perhaps the most notable instance of selectivity.

Penicillin is produced by the growth of *P. notatum*. It inhibits strongly the growth of most of the Gram-positive cocci, some of the Gram-negative cocci, and some Gram-positive bacilli, while it has practically no inhibitory action on the coliform group, the hæmophilus group and the Brucellæ. This makes it valuable as a differential bacteriostatic agent, and by its use it has been shown that bacteria of the *B. influenzæ* group are present in 100 per cent. of the mouths of healthy people. By the use of penicillin it is possible to divide the Gram-negative cocci sharply into two groups : gonococcus, meningococci, and *M. catarrhalis* are all sensitive, while *M. flavus* and other non-pathogenic cocci are insensitive.

Potassium tellurite exhibits almost opposite bacteriostatic properties to penicillin. *B. typhosum* is inhibited by a dilution of about 1 in 10,000,000, while *C. diphtheriæ* grows in a concentration of about 1 in 1,000. Some bacteria such as the enterococcus, some leptothrices, yeasts and moulds are resistant to both penicillin and tellurite. Very selective media can be prepared by incorporating various dilutions of penicillin or tellurite or both.

Attention is directed to the selective action of mercuric salts on hæmolytic streptococci in blood.

It can be shown that there is complete bacteriostasis of hæmolytic streptococci in fresh human defibrinated blood containing from 1 in 50,000 to 1 in 100,000 of mercuric chloride, whereas if the concentration of the salt is 1 in 20,000 the streptococci will grow out. If the leucocytes are removed from the blood this bacteriostatic action disappears. This bacteriostasis is a combined effect of the mercury salt and the leucocytes and is probably due to a lengthening of the lag period of the cocci, thus allowing time for the leucocytes to destroy them before they have commenced to grow out. Actions of this kind may be of great importance in chemotherapy.

A study of selective bacteriostasis is important in many ways, notably in the preparation of culture media which will eliminate the unwanted contaminants of a mixed implant. In chemotherapy, also, it is a subject of extreme importance, but in this connexion tests must be made in blood or some other medium which reproduces to some extent the conditions in the animal body.

*__Prof. F. Eisenberg__ (Cracow): *Selective Bacteriostasis and Bactericidal Action.*

"Selective" means either the different inhibitory or bactericidal effect of various substances on the same germ or the different influence of one substance on various groups, species, varieties, or individual strains. Experiments* with 836 substances—217 inorganic, 502 organic, 117 aniline dyes—have given the following results.

Toxicity for bacteria can be regarded as an additive function of ions. The anion series of toxicity is:

$SO_4 < S_2O_3 <$ Tart. $< H_2PO_3 < MoO_4 < Cl < Br < NO_3 < SO_3 <$ Fe/CN$_6$'''' $<$ Ac $< ClO_3 <$ Citr. $< HPO_3 <$ Form. $< SCN < ClO_4 < BrO_3 < I < H_2PO_4 <$ Benz. $<$ Nitropr. $< HAsO_4 < CrO_4 < P_2O_7 < NO_2 < F < BF_4 < B_4O_7 <$ Fe/CN/6''' $<$ Sal. $< HSO_3 < IO_4 < S_2O_8 < S_2O_7 < TeO_4 < SbS_4 < OsO_4 < IO_3 < Cr_2O_7 < TeO_3$

The cation series runs:

Na $<$ K $< NH_4 <$ Li $<$ Mg $<$ Sr $<$ Ca $<$ Ba $<$ Mn $<$ Th $<$ Fe $<$ Y $<$ Cr $< UO_2 <$ Zn $<$ Fe $<$ Ti $<$ Be $<$ Al $<$ Nd $<$ Pb $<$ Cu $<$ Tl $<$ Zr $<$ Ni $<$ Cd $<$ Co $<$ Au $<$ Pt $<$ Hg $<$ Ag

In the organic series bacterial toxicity was found to be influenced by the chemical structure, the quality, quantity and position of the groups, the molecular volume, lipoid solubility, cohesion,

capillary activity, diffusibility, internal friction, but the most important character is doubtless their activity on the bacterial biocolloids as influenced by the electrochemical charge. The greater their germicidal potency, the more they provoke a swelling or a shrinkage of bacterial protoplasts.

The " Gram-selectivity " of germicides is either of the dye-type, i.e., the Gram-positives are more influenced than the negatives, or of the inverse type, when the reverse is true. The aniline dyes, the cadmium, zinc and mercury salts are representatives of the first type; chromates, bichromates, inorganic and organic basic substances and many lipoid solvents, of the latter. It is supposed that the Gram-positives are more sensitive towards coagulants, the negatives towards swelling factors. Applications to the selective culture of germs and viruses and to chemotherapy of bacterial infections were discussed.

Dr. I. Forest Huddleson (East Lansing, U.S.A.) : *The Mechanism of the Bacteriostatic Action of Dyes on the Growth of the Brucellæ.*

The phenomenon of dye bacteriostasis and of its practical utilization in the identification of the species of a newly isolated culture, is strikingly shown by the behaviour of the Brucellæ toward basic fuchsin, pyronin and thionine in liver infusion agar.

Since many attempts have been made to connect the bacteriostatic action of dyes with their ability to poise the oxidation-reduction potentials of media at a level unfavourable for bacterial growth, it was decided to explore this field when seeking an explanation for the selective bacteriostatic action of dyes. Experiments were therefore performed to determine first, the oxidation-reduction potentials associated with the growth of the three species of Brucella; second, to determine the effect of the addition of the dyes on their oxidation-reduction potentials.

The electrode potentials in these experiments were measured under aerobic conditions. This requires the employment of a system in which the potential of the cell will remain at or near the same level for a period before inoculation. The maintenance of electrode potentials at a constant level in liquids containing organic materials has been difficult without the introduction of some dynamic method to prevent the drift of potential and the polarization of the electrode. These troublesome factors were met by using a large electrode made of Acheson special graphite.

The experiments revealed the following information: There were found little, if any, differences between the rate of reduction or final Eh level reached by *Brucella abortus*, *Brucella suis* and *Brucella melitensis* in liver infusion broth without dyes.

In the presence of thionine in a final dilution of 1-30,000, it was observed that the reducing intensity of *Br. abortus* was inhibited altogether. In the presence of basic fuchsin in a final dilution of

1-20,000, *Br. suis* causes little or no drop in the initial Eh of the medium. The presence of the two dyes in the broth does not retard the reducing intensity of *Br. melitensis*.

In so far as defining the growth activities of each of the three species are concerned, the electrode potentials recorded in these experiments parallel the visual findings on a solid medium to which the same dyes have been added and inoculated with Brucella.

The results of these experiments, however, do not show that the selective inhibiting action of the dyes is directly related to a stabilizing effect on the oxidation-reduction potential of the medium for the following reasons :—

(1) The Eh of an aqueous solution of thionine and basic fuchsin in an oxidized state is less than that of the sterile liver infusion broth used in this study either with or without dyes.

(2) The addition of the dyes to the medium caused no measurable changes in the potentials of the cells over the potentials observed in those without dyes.

(3) There was noted no initial reduction of the potentials of the cells after inoculation to the point where the poising action of the dyes interferes with further metabolic activity.

(4) The final Eh levels that obtained in the inoculated cells with and without dyes at the end of the nine-day incubation period were little, if any, lower than those of the dyes when measured in the oxidized state.

Data obtained from a large number of observations and experiments by the writer, indicate that when a dye is added to culture media, a chemical or physical union occurs between the dye and certain food substances in the media necessary for bacterial growth. When united with certain dyes in the proper concentration, the food substances cannot be utilized for growth energy by Brucella. Thus, there follows no visible growth on the medium. It has been observed that if Brucella grows well on a solid medium containing either basic fuchsin or thionine, the surrounding medium slowly becomes decolorized due to a gradual withdrawal of the dye from the medium. The dye appears to be stored on or within the bacterial cells. This is evident from the intensity of the colour of the visible growth on the surface of semi-solid medium. As the growing bacterial cells withdraw food from the semi-solid medium for growth energy, the dye in combination with the food is also withdrawn from the medium and concentrated in or on the bacterial cells, the colour of the bacterial growth becoming intensified while that of the medium is diminished.

The mechanism of dye bacteriostasis in the case of Brucella would appear to lie in inherent differences in the capacities of certain of the species to assimilate for growth energy the necessary food

substances that are combined with certain dyes in the proper concentration.

Dr. L. P. Garrod (London) : *The Bacteriostatic Action of Gentian Violet.*

Since Churchman's original demonstration that susceptibility to the bacteriostatic action of gentian violet varied with the Gram staining reaction, this property had been studied in greater detail, and minor differences had been observed among Gram-positive bacteria, some of which, such as the greater susceptibility of staphylococci than streptococci, could be turned to useful account. The results were given of a detailed investigation of the susceptibility of Gram-positive cocci, in which multiple strains of each organism were used and closely spaced dilutions of five different violet dyes. The concentration inhibiting all staphylococci was markedly less than that preventing the growth of the pneumococcus or any of three types of streptococcus. A similar study embracing a wider variety of bacteria showed that species of the genus *Bacillus* were even more susceptible than the Gram-positive cocci, whereas species of *Bacterium* showed a varying but hitherto unsuspected degree of resistance, *Bact. paratyphosum B.* growing even on a medium containing 1 part in 296 of the dye and remaining viable in spite of being deeply stained by it. There was a 6,000 fold difference in degree of resistance between this organism and some Gram-positive bacilli. The mode of action of the dye was unknown ; if, as had been suggested by Dr. Huddleson, it interfered with nutrition, this effect could only satisfactorily be studied in culture media of exactly known and simple composition.

Dr. P. Fildes (London) : *The Bacteriostatic Action of O/R Dyes.*

It has been reported that O/R dyes more positive than indigotetrasulphonate in the oxidized state inhibit the growth of aerobes, and that this is an indication that even aerobes require a low Eh in the medium to grow.

It has, however, been found that only diffusible dyes (non-sulphonated) cause this inhibition when oxidized, and the same applies to diffusible dyes below the level of indigotetrasulphonate. The more positive indophenols do not inhibit because they are reduced even aerobically. The inhibition is only observed when the concentration of the dye is high.

The phenomenon is thought to have no relation to the Eh of the medium, but to be due to the oxidation of some compound within the cell which requires to be reduced in growth.

***Prof. W. Brown** (London) : *The Production of Growth-inhibiting Substances by Fungi.*

By the growth of a fungus in a culture medium, the latter finally becomes incapable of supporting growth or inducing germination

of fungal spores. This stage is in general reached before the food constituents of the medium are exhausted, so that the presence of growth-inhibiting substances is indicated. With the same fungus the nature of the "staling" substances varies according to the make-up of the medium, and in particular according to the type of nitrogenous substance provided. In the case analysed the medium became alkaline without evolution of ammonia. The staling factor was not alkalinity as such, but an alkaline *p*H due to bicarbonate. The latter, for a given *p*H, is much more actively repressive of growth than is carbonate or hydroxide. The behaviour of staled media on boiling, acidification, or fractionation with ether is readily interpreted. The staling of a medium by accumulation of bicarbonate is inevitable if the nitrogenous food constituent of the medium is such as to furnish a basic ion, the latter combining with the carbon dioxide of respiration to produce bicarbonate.

Prof. W. J. Wilson (Belfast): *Selective Bacteriostasis effected by Metalloids alone or with Sodium Sulphite.*

My remarks deal with the selective inhibitory action of bismuth and arsenic.

The bismuth sulphite medium (Wilson and Blair) is based on two observations of mine—(1) that in the presence of a fermentable carbohydrate certain organisms, members of the *Eberthella, Salmonella* and *Clostridium* genera, have the power of reducing a sulphite to a sulphide. (2) That when a bismuth salt is present as an indicator for the reduction, the growth of *Clostridium* and *Escherichia* genera is inhibited, but there is little impairment of the growth of the *Proteus, Eberthella* and *Salmonella* genera.

With a view to throwing light on the factors concerned in the bacteriostasis, a fluid medium has been studied—Broth 1000 c.c. *p*H 7·2, containing 10 g. peptone (Difco), 5 g. Lemco or, instead of Lemco, 0·5 g. sod. phosphate anhydrous. To 1000 c.c. of the fluid medium are added 10 g. sod. sulphite anhydrous and 2 c.c. Liq. Bis. et Ammon. Cit. (B.P.). On boiling, a precipitate of bismuth hydrate falls. On analysis, the sulphite does not appear to be reduced in the solution and the precipitation is due to the alkaline reaction *p*H 8·4. The clear fluid above still contains a trace of bismuth in solution, and this amount is sufficient in the presence of sodium sulphite to act bacteriostatically and continues to act when left in contact with the bismuth hydrate precipitate.

The *p*H is important; if the *p*H of sulphite solutions (without bismuth) is reduced to *p*H 7·6, fæcal *B. coli* grow, but strains of *B. aerogenes* are inhibited.

In the bismuth sulphite medium the reducing action and solvent action of the sulphite on bismuth salts, a suitable *p*H, and the presence or absence of agar, are important factors.

Arsenic. If to 100 c.c. blood agar medium 2 c.c. of 1 per cent. sodium arsenite are added, diphtheroids are inhibited and the

majority of strains of *C. diphtheriæ* grow. This can be combined with the addition of 2 c.c. of a 1 per cent. solution of sodium tellurite.

Dr. T. K. Walker (Manchester): *The Effects of Bacteriostatic Compounds on the Growth-time Curves of Bacterial Proliferation.*

Determinations have been made of the growth-time and acidity-time curves of several species of lactic acid-producing bacteria when cultivated under standard conditions on maltose media in the absence and in the presence of different chemical bacteriostatic substances.

Under normal conditions of growth a logarithmic phase in the acidity-time curve was noted which corresponded almost exactly with the logarithmic phase of the growth-time curve, hence during the logarithmic phase determinations of acidity provide a measure of the rate of reproduction.

The bacteriostatic compounds in quantities insufficient to cause total inhibition of proliferation were added at inoculation and also at various stages of the lag and logarithmic phases of growth. The maximum retardation of rate of growth caused by a given quantity of substance was observed when such dose was added at the commencement of the logarithmic phase, and was manifested as a regular but lower rate of acid formation representing a regular but lower rate of bacterial reproduction, as compared with the corresponding rates in control experiments. Based on this observation a method was elaborated, by the author and J. J. H. Hastings, for the quantitative comparison of the bacteriostatic potencies of very small quantities of substances. *L. bulgaricus* was cultivated in flasks of malt-wort under rigidly controlled conditions. At the commencement of the logarithmic phase doses of the substances to be compared were added to the several flasks. At the end of the logarithmic phase the acid in each flask was titrated and the percentage restriction was calculated from comparison with a blank. For each substance the graph of percentage restriction plotted against quantity of antiseptic was found to be a straight line between certain limits of restriction. These limits differed with each substance. Usually, the extension of this straight line intersected the abscissa at a distance from the origin which varied with the particular antiseptic. The expression for such a graph is $y = mx + c$, hence, if percentage restrictions are denoted along the ordinate and quantities of antiseptic along the abscissa, it follows that: Percentage restriction $\div$ (Weight of antiseptic which produces that restriction $- k$) $=$ K, where k is the weight of antiseptic represented by the distance between the origin and the point at which the prolongation of the linear portion of the graph cuts the abscissa. The values of both k and K are fixed for any one antiseptic, but vary from one antiseptic to another. In certain cases (*e.g.*, phenol) the graph passes through the origin, hence, in

such cases, $k = 0$. The method is of value for estimating the potencies of small amounts of antiseptic material in dilute aqueous solution, for example in infusions of hops.

DISCUSSION *on* PRESERVATION OF CULTURES OF MICRO-ORGANISMS. — LATENCY. METHODS OF PRESERVATION OF DELICATE ORGANISMS. PRESERVATION OF VIRULENCE AND ANTIGENIC STRUCTURE. CHANGES IN THE CHARACTER OF BACTERIA IN CULTURE MEDIA UNFAVOURABLE FOR RAPID GROWTH

Chairman : Prof. E. Gotschlich (Ankara).

Dr. Homer F. Swift (New York) **Opener** : *The Preservation of Bacterial Cultures by Freezing and Drying.*

That frozen and dried bacterial cultures and viruses maintain their original states of virulence and invasiveness, and their immunochemical properties has been demonstrated by numerous experiments. Hæmolytic streptococci have been tested in this respect up to twenty years. Pneumococci, meningococci and other delicate bacteria have been shown to maintain very labile properties when preserved in this way. Such filterable viruses as those causing Rous sarcoma and yellow fever have been similarly preserved ; also labile antigenic material separated from intact bacteria. Freezing at $-76°$ C. has been shown to be an effective method of preserving *Treponema pallidum* (Turner) ; probably rapid freezing at $-76°$ C. is less injurious to protoplasm than slower freezing at $-6°$ to $-10°$ C. Simple drying of bacteria on paper, or on inert particulate matter is sufficient to preserve some bacteria, especially in the presence of some protective colloid such as serum, or defibrinated blood : but the duration of viability is irregular and cannot be predicted.

The abstraction of water from frozen bacteria or other material prevents a harmful concentration of injurious agents. Freezing may be effected by placing containers (*a*) in salt-ice mixtures, (*b*) in the freezing compartments of mechanical refrigerators, (*c*) in CO_2 ice-alcohol baths, (*d*) by rapid evaporation in a high vacuum. Drying may be attained (1) by placing some highly hygroscopic chemical in the vacuum system, (2) by low temperature distillation, or sublimation of the water vapour, or (3) a combination of the two. Method (2) is utilized in the apparatus of Flosdorf and Mudd ; method (3) in that of Elser, Thomas and Steffen. These are especially applicable in the preservation of large amounts of material such as sera or other biological products, and method (2) has the additional advantage of permitting the tubes to be sealed with the original vacuum.

Method (1) has been employed extensively by the author, for preserving many small tubes of bacterial culture. Since CO_2 ice (dry ice) has become readily available his original method has been

simplified. It is recommended that the sediment from broth cultures, or from the surface growth on solid medium, be suspended in small amounts of serum or serum broth, and placed in sterile cotton-stoppered tubes about 8 × 120 mm.

The desiccator contains glycerol in which the tubes are immersed, and above them is placed a flat dish of the desiccant, P_2O_5. By placing CO_2 ice in the glycerol the concentrated cultures can be frozen in the desiccator; the air is then evacuated, and the pump kept running for at least 30 minutes after the CO_2 is completely vapourized. The desiccator must be kept at a temperature below 0° C. to prevent melting of the cultures until drying is complete. This is easily done if it has been placed in a cork box containing a sheet metal box of CO_2 ice. Drying is usually completed overnight. Admitting dried air or nitrogen into the desiccator before opening is advisable. The tubes may be secondarily evacuated and sealed in a flame, or, more simply, by pouring hot wax into the space made by depressing the cotton plug about 5 mm. Paraffin wax is an insecure seal; sealing wax is better, but is liable to crack at low temperatures; " Piscein " is the best so far tried. Where CO_2 ice is not available, freezing may be accomplished by immersing the tubes and desiccator in a salt-ice mixture.

Properly preserved material has a whitish flaky or porous appearance; improperly dried material, or that in which the seal is broken, and moisture admitted, has a yellowish gummy aspect.

Dr. W. J. Elser and Dr. Ruth A. Thomas (New York): *The Preservation of Bacteria in an Unaltered State for an Indefinite Period.*

Our work in this field, which dates back to 1909, developed out of experiments then under way directed toward the perfection of a method for the preservation of immune sera. It was not until 1912 that the methods used with success for immune sera were applied to the preservation of such sensitive bacteria as the meningococcus and gonococcus. In 1916, the experiments were repeated with the meningococcus and gonococcus and both were found to be viable eighteen years later.

Following the method devised for the preservation of immune sera by drying in the frozen state and storage *in vitro*, manifolds containing the drying agent were originally used for the desiccation of bacteria. For the past six years an apparatus designed especially for bacterial work has been employed. By its use the number of manipulations involved is greatly reduced and the number of tubes that can be dealt with conveniently is materially increased. Suspensions dried in this manner differ markedly in texture and in appearance from those dried in the liquid state. They are not scaly but retain the shape and approximate appearance of the frozen material with no appreciable reduction in volume. To prove that the original characteristics of dried bacteria are also preserved,

comparative studies of fifty strains, frozen and dried by the above method and stored *in vacuo*, were begun in 1923. The morphological, cultural and biochemical characteristic of each strain were determined at the time of storage, after six months and then annually for a period of four years. Control tests were carried out on the same strains kept in semi-solid media during this period. In each instance the strains stored by this process maintained their original characteristics, while those in semi-solid media sometimes showed morphological and cultural variations. Subsequent tests with the pneumococcus have shown that virulence remains constant and with the anaerobes that toxin production is unimpaired. *B. pyocyaneum* maintained its ability to elaborate pyocyanin, while corresponding cultures on artificial media lost this property in a few months. Bacteria can probably be maintained in a viable state indefinitely by this method. We have recovered meningococci and gonococci in pure culture after storage *in vacuo* for eighteen years. Experimental evidence obtained in connection with other work also shows that the behaviour of these strains toward immune sera remains unaltered when they are recovered on suitable culture media. For the past fourteen years we have preserved all the cultures used for teaching purposes in this manner. This has eliminated the necessity of making monthly transfers and also the risk of losing valuable strains through death or contamination. We have also employed this method in the preservation of all of the strains used in our various research problems and have found it particularly valuable in our work with bacterial types and mutants.

The method provides a means for the preservation in an unalterable state of cultures used in the manufacture of prophylactic vaccines and might serve for the standardization of such products.

This method of preservation of bacteria should open the way to the establishment of a museum of bacteria which has genuine value. Collections are maintained in various countries, but are of doubtful worth since strains kept on artificial culture media tend to lose some of their most characteristic peculiarities. It should also reduce the cost of maintenance of museums and make possible the successful shipment to any part of the world of even the most sensitive strains.

We have successfully preserved five wine yeasts in this manner, but to date our attempts to preserve protozoa and spirochætes, though numerous, have been discouraging. *Spirochæta duttoni* was recovered once and *Trypanosoma brucei* twice, but in both instances immediately after freezing and drying. We have also attempted to preserve malarial parasites but without success. However, further efforts in this direction with various modifications of technique in the preparation and recovery of the suspensions are contemplated.

Dr. R. St.John-Brooks and **Miss Mabel Rhodes** (London) : *Some Useful Media for the Preservation of Stock Cultures. Notes on High Vacuum Desiccation.*

The following media have been found of considerable use in the routine subculture of micro-organisms in the National Collection of Type Cultures. In all cases the media should be steam sterilized.

Cane Sugar Solution. Ten per cent. sucrose is dissolved in distilled water. A loopful of a yeast culture or a small piece of agar containing spores and mycelium of an actively growing fungus culture is used as inoculum. Yeasts and the majority of fungi remain typical and are viable for at least a year. In the case of *Aspergillus* and *Penicillium* spp. growth may be obtained after from two to five years.

Neisseria Medium. Semi-solid serum agar made with Difco proteose peptone is tubed and topped with 1 ccm. liquid paraffin. In this medium *N. intracellularis* is viable after from one to two years, *N. gonorrhœæ* after from six months to one year, and *Streptobacillus moniliformis* after six months.

Vibrio Medium. Ordinary nutrient agar diluted with tap water to 0·2 per cent. is employed. All vibrios tested (including *V. choleræ*) were alive after one year.

Potato Agar Medium. 200 grammes of potato and 15 grammes of agar are added to one litre of tap water. Spore-bearing aerobes spore very freely on this medium and keep alive almost indefinitely.

Recently an extensive trial has been given to drying cultures *in vacuo*. Experience with high vacuum desiccation over phosphorus pentoxide, as recommended by Sordelli, has on the whole been favourable ; with improved technique better results may be expected. After periods varying from one year to 18 months, types of the following organisms were found to be viable : *Hæmophilus influenzæ, Actinobacillus lignieresi, Diplococcus pneumoniæ, Vibrio choleræ, Neisseria catarrhalis, N. intracellularis, N. pharyngis, Actinomyces bovis* (Wolff and Israel), *Streptococcus hæmolyticus, Brucella abortus, Bacterium lathyri* and other phytopathogenic organisms. In cases where tests have been made, the organisms have remained typical and of unimpaired virulence.

Dr. A. C. Thaysen (Teddington, England) : *The Preservation of Stock Cultures.*

In 1924 the writer first reported on two methods for the preservation of type cultures : (1) based on the observation that lactic acid bacteria can be kept alive for a long period when calcium carbonate is added to their cultures, (2) the use of washed and sterilized sea-sand for the purpose of storage.

He has also reported on the results of the examination of a series of organisms kept stored at room temperature either in their usual media after addition of a small quantity of calcium carbonate, or

on washed, sterilized sand in a dried condition. The latter method was used only for spore-forming types. With one exception all the types chosen for the tests and stored in the presence of calcium carbonate remained alive without subculturing for one year, and some of the cultures for periods up to twelve years.

Of the four spore-forming rods stored on dry sand all had remained alive when tested twelve years later.

Particular interest attaches, perhaps, to the results obtained with *Thiobacterium thiooxidans* of Waksman and Joffe. This organism can be kept alive in the laboratory only by frequent subculturing, but when stored in the presence of calcium carbonate, has so far remained alive without subculturing for six months.

As the procedures are very simple, a brief account of them may be given.

(1) Calcium carbonate—to each 2 ml. of a young, vigorous, liquid culture about 0·2 g. of sterile precipitated calcium carbonate is added aseptically and the test tube in which the culture is contained sealed off.

(2) Sand—approximately 5 g. of clean, washed sea-sand is sterilized in a test tube. After cooling, 2 to 3 drops of a previously prepared spore-containing culture are placed on the sand, which is then gently shaken until uniformly damp. The tube is placed in an incubator at 37° C. until dry, when it is sealed off and kept at room temperature.

Mr. S. F. Ashby (Kew) : *Survival in Culture of a Sensitive Fungus—* Phytophthora hibernalis *Carne.*

This species is a pathogen of citrus (especially of the sweet orange) and is now recognized as of wide distribution—Portugal, Western Australia, Victoria, California and Cape Province, South Africa. It infects the leaves and young shoots and causes a brown-rot of the fruit during the cold, rainy period towards the end of the winter. The optimum temperature for growth is between 15 and 20° C. but falls off rapidly above 20° C. ; there is no growth at 25° C. and the mycelium dies quickly at that temperature, and is difficult to keep alive in pure culture during the summer. On suitable solid substrata oospores and sporangia develop readily but the sporangia are thin-walled and soon lose their vitality at and above 25° C.

In January, 1933, two isolations, one from California and the other from South Africa, were transferred to oatmeal agar slants in tubes and allowed to grow at about 15° C. After a month growth had ceased and a number of oospores had matured. The cultures stood at the temperature of the laboratory during the prolonged drought of 1933 with spells of very high temperature during summer. In January, 1934 a request was received for cultures of this species ; the tube cultures, just a year old, were then partly dried out and the prospect of obtaining growth from them seemed remote. They

were flooded with sterile water and put into a cool room; after 10 days short tufts of mycelium had grown into the water from the surface of the agar at a number of points. The water was poured off and the young mycelium stroked with a bent platinum needle and transferred to plates of maize-extract agar. After a week at about 15° C. pure colonies of *P. hibernalis* had developed on the plates from both of the cultures. It was evident that a number of the oospores, matured during January of the preceding year, had survived the prolonged high temperature of the summer of 1933 and had retained the power to germinate after a year.

Under field conditions survival of the species from one winter to the next doubtless occurs in the same way by means of resistant oospores; these are known to be produced in the rind of infected fruit which tends to fall prematurely. *P. hibernalis* has not been recorded on citrus from within the tropics where the cool season is dry and the summer wet, the reverse of the conditions which favour its activity.

Dr. E. W. Flosdorf and **Dr. Stuart Mudd** (Philadelphia): *Rapid Drying of Serum and Micro-organisms from the Frozen State for Preservation.*

For optimal conditions of drying unaccompanied by protein denaturation, rapid drying as well as the rapid initial freezing at − 76° C. mentioned above by Dr. Swift is necessary. Rapid drying serves also to provide the only means of cooling required as well as automatic temperature change of the drying material. As quickly as possible the product reaches room temperature thereby producing in the final stages a very high drying-potential.

To achieve this rapid rate of drying, our procedure[1] reqnires large bore exhaust tubes and that the customary cotton plugs be removed from the ends of these exhaust tubes at the moment they are pointed downward for insertion into the manifold outlets. Asepsis is maintained as a result of (1) the "L" shape of the exhaust tube in combination with the angle of the outlets on the manifold and (2) the rapid outward flow of air and vapours.

The condenser construction of our apparatus is critical. The main condenser, in order to collect large volumes of condensate without clogging, cannot be made more than 99·5 per cent efficient. The very efficient secondary condenser therefore is required to protect the pump. Clogging of the secondary condenser occurs only if there is a leak, improper packing of the bath, or other improper manipulation and by clogging will protect the pump from ill-effects resulting therefrom.

Dr. R. B. Haines (Cambridge).

Aqueous suspensions of bacteria were frozen rapidly at − 70° C. and stored at various temperatures such as − 1, − 2, − 5, − 10

[1] *J. Immunol*, 1935, 29, 389.

and — 20° C. Viable counts were made on the tubes. At — 20° C. the rate of death was slow, and at temperatures round — 3° C. comparatively rapid. There was no significant difference in viability with the rate of freezing, so that size of ice crystals will not account for the phenomenon. It is known from work initiated by the late Sir William Hardy and carried on by Dr. Moran and others that in the storage of muscle there is a critical temperature at — 2° C. at which optimum denaturation of certain of the cellular proteins occurs. This denaturation is due to the particular salt concentration obtained at that temperature. I think there is little doubt that a similar phenomenon is taking place with bacteria. The use of low temperature freezing thus depends on taking the organisms rapidly through its critical temperature, and once dry they can be left for long periods at room temperature. *B. coli* for example has been kept viable for at least two years at — 20° C., but is dead in a few weeks at — 2° C., in the frozen state. No difference was obtained with spores of *B. mesentericus* or *B. megatherium* when stored at — 2 and — 20° C.

Prof. E. Gotschlich (Ankara).

Zu den sehr interessanten und für die Laboratoriumspraxis wertvollen Mitteilungen der verehrlichen Vorredner über die ausserordentlich lange Lebensfähigkeit von Bakterien unter Kälte und Luftabschluss möchte ich einige Bemerkungen allgemein-biologischer Natur hinzufügen, die sich mit der Ursache dieser auffallenden Erscheinungen befassen. Es handelt sich im wesentlichen darum, dass unter genannten Versuchsbedingungen das Leben in latenten Zustand übergeführt wird, in dem die Dissimilationsprozesse zurücktreten und demgemäss die langdauernde Erhaltung des Lebens möglich ist. Diese grundlegende Tatsache ist schon seit etwa 40 Jahren bekannt; zuerst wies Petruschky nach, dass Streptokokken, in Stichkultur im Eisschrank konserviert, lange lebend und virulent bleiben, während sie in Oberflächenkulturen bei Zimmer-oder Bruttemperatur rasch absterben. Den Absterbevorgang konnten bald darauf E. Gotschlich und G. Weigang zahlenmässig exakt verfolgen; eine Oberflächenkultur von Choleravibrionen auf einen Schrägagarröhrchen erreicht das Maximum ihrer Entwicklung mit etwa 30 Milliarden Keimen schon nach 12–24 Stunden, während nach weiteren 24 Stunden bei Aufbewahrung im Brutschrank die Zahl der lebenden Keime nur noch eine Milliarde beträgt und in den nächsten Tagen rapid weiter absinkt, bei Eisschranktemperatur hingegen die Keimzahl längere Zeit konstant auf derselben Höhe bleibt, die dem Stadium der Entwicklung entspricht, das bei Überführung der Temperatur vom Brutschrank zum Eisschrank erreicht war.

Mr. C. E. Coulthard (Nottingham).

During studies of the resistance of mixed bacterial spore suspensions to heat treatment (Tyndallisation), it had been found

that spore suspensions in dilute glycerin solutions maintained an even resistance over long periods. Glycerin, at all events in concentrations up to 30 per cent., did not appear to affect the resistance at 80° C. and spore suspensions in 30 per cent. v/v glycerin had shown no diminution of resistance in 6 months whether stored at 4° C. or 37° C. A suspension in 15 per cent. glycerin had been tested over a longer period and had shown no apparent loss of resistance after 12 months.

Mr. L. B. Holt (London) : *The Comparative Efficiency of Different Methods of Preserving Staphylococci for their Toxigenic Properties.*

Six strains of toxigenic staphylococci were preserved in ten different ways for a period of one year.

The desiccation method was a simple one ; 10–15 cmm. of material were placed in very small tubes, rapidly dried under P_2O_5 and sealed under a vacuum.

Results.	Toxigenic Property.*	Variants Formed.	
		Toxigenic.	Atoxic.
Method.			
1. Dried in whole blood ...	× × ×	0	0
2. Dried in serum	× × ×	0	0
3. Dried in broth	× × ×	0	0
4. Meat under paraffin, subcultured 6 weeks	× × ×	6	0
5. Meat under paraffin, 12 months	× +	0	0
6. Nutrient agar under paraffin, subcultured 6 weeks ...	× ×	4	3
7. Blood—agar slope, subcultured 4 weeks	× +	0	0
8. Nutrient agar slope, subcultured 4 weeks	×	2	5
9. Broth, subcultured 4 weeks	×	5	7
10. Egg, subcultured 6 weeks...	× —	3	6

The desiccation methods were best, of these desiccation in serum gave the most favourable results.

* × × ×. Toxigenic property preserved.
× ×. „ „ impaired.
×. „ „ greatly impaired.

DISCUSSION *on* LIFE-CYCLES OF BACTERIA. SYMBIOTIC ASSOCIATIONS. FILTERABLE FORMS

Chairman : Prof. E. Gotschlich (Ankara).

Dr. J. Ørskov (Copenhagen), **Opener :** *On some Extracellular Elements in Bacterial Cultures giving rise to " Colony-formation."*

I shall, in the main, confine myself to some few morphological features upon which I have been working during recent years. These are certain formed extra-cellular elements which under given conditions appear in some bacteria cultures. I cannot, however, entirely disregard a question which has often been asked in connection with these deviating forms, namely, the important factor as to whether it is not possible that the monotony usually seen in bacteria cultures is merely apparent ; whether a much more complicated developmental process cannot take place simultaneously, for example, with a growth of elements so minute that they escape detection by the ordinary investigatory methods, elements which might be of paramount importance both as to systematic bacteriology and in a pathogenetic sense. We have an illustration of a true developmental cycle, for instance, in the actinomyces group. We have here fungi which produce spores. The spores grow into a unicellular mycelium which produces spore-threads, and these in turn divide into spores capable of passing through the same process of development.

Or take, for instance, a microbe such as the pleuropneumonia virus, where we have a terminal stage, a small oval or globular body which first grows into an undivided, true mycelium, which then separates into single elements, the terminal forms, capable of undergoing development in the manner just described.

If we turn to bacteria proper, we find that it has often been maintained that they also undergo similar, or even much more complicated, stages of development. In brief, I may say that no one has ever demonstrated with absolute certainty such developmental cycles in genuine bacteria, and that the cause of interpretations in the opposite direction has usually been the use of unsatisfactory technique. In connection with the question of technique, I believe that I am justified in stating that the more direct the method can be made, the less possibility there is for interpretations of a speculative nature.

In all my experiments I use agar microscopy as the fundamental method. The technique consists simply in examining by means of suitable magnification the growth of the bacteria on the surface of the solid, transparent culture medium.

Another technical aid, used extensively in these experiments, where one frequently has to do with invisible elements, was India ink. This was used as a contrast—sometimes in smear preparations, sometimes combined with direct agar microscopy.

With regard to details, I would refer to the comprehensive descriptions already published.

In the following account I shall confine myself to certain peculiar extra-cellular formations which arise when certain bacteria grow on culture media containing saccharose.

It has long been known that Leuconostoc- or Betacocci-strains produce mucus on media containing saccharose, usually in the immediate vicinity of the bacteria. Chemically the mucus is mainly composed of polysaccharides. It is generally only loosely attached to the bacteria, and consists partly of an easily stained, threadlike substance, and partly of non-stainable, irregularly shaped bodies, which may be rendered visible by indian ink.

Here I propose to deal only with the non-stainable part of the mucus. The mucus produced by most Leuconostoc-strains does not develop in colonies which do not contain bacteria.

Many of the streptococci generally found in the human throat can produce excessive amounts of polysaccharide when grown on media containing saccharose. The elements making up the pasty mass are non-stainable and so small that they cannot be rendered visible by the indian ink method. The substance is produced only in and around the colonies of bacteria.

Several strains of actinomyces belonging to the author's group II.b., cultivated from samples of Danish soil produce polysaccharide in excess on saccharose media. The elements in the mucus are polymorphous. There is no independent colony-formation of these elements.

Among the Leuconostoc-strains are those showing a peculiar bacteria-free formation of "colonies" in and around the colonies of bacteria. These "colonies" are partly made up of small unstainable elements composed chiefly of polysaccharide. The agent apparently diffuses in the solid media producing mucus in the form of "colonies." These "colonies" never arise primarily on media by subcultivation from cultures containing the mucus.

Dienes in his study of strains of *B. subtilis* which grow "slimy" on saccharose-media, found that the mucus or agent producing it may develop independently of the bacteria after subcultivation for a few "generations." The agent causing the colony-formation is filterable, and is probably primarily produced by the bacteria.

Lastly there is a group of Gram-negative, motile bacilli first found in milk, and later often in samples of soil, which on saccharose media show excessive production of extracellular, non-stainable bodies of very uniform size and shape, easily demonstrable by the indian ink method. The agent responsible for their development is probably produced by the bacteria. It can be found free in cultures which do not contain saccharose, and can produce "colonies" composed of the bodies and free from bacteria. This agent cannot diffuse through the solid media. Subcultivation has sometimes been successful for one or two "generations."

The bodies themselves cannot represent the agent (the causative substance), which is probably corpuscular, and is filterable. It is more resistant to heat and chemicals than the bacteria. I have termed the agent a virus on account of the difficulty in attributing the phenomena to a fermentative process, although I consider that the term is not happily chosen since we do not know what a virus really is.

There was a time when I thought it might be a question of a sort of symbiosis between the small unstainable elements and the bacilli. To-day I believe there is no doubt that the small elements are formed by an agent whose origin is to be found in the bacilli. The agent is also found in cultures without saccharose. This can be illustrated by inoculating such bacillus cultures, for by that means we can show primary development of bacteria-free colonies. These remain bacteria-free throughout their entire development.

Dr. L. Dienes (Boston. U.S.A.): *Production of Amorphous Substances in Bacterium Cultures suggesting Growth Phenomena not connected with Bacterial Forms.*

Ørskov had observed that the amorphous extracellular substance, which is present in many bacterium cultures, in certain cultures in the presence of saccharose shows phenomena which suggest independent growth without direct influence of the bacteria. The amorphous substance spreads out from the colonies on the surface of the medium and forms a halo similar to that of a spreading proteus colony. In other media no halo forms, but tiny colony-like masses develop inside the medium. Transplants from the halo grow as tiny colonies consisting of a similar amorphous substance. Formation of colonies was observed in four consecutive transplants. The properties of the halo strongly suggest that it is formed by growth of living elements.

The growth of the amorphous substance is most abundant on non-filtered agar containing no other nutrient substances except saccharose. I have found only two substances, silica and killed fresh cultures of certain moulds, which increased the growth, and this effect is specific. The formation of amorphous substance proceeds through two consecutive phases which are most easily visible in dextrose agar cultures. From the colonies a non-visible agent diffuses into the medium, and if the medium is covered later with a concentrated solution of saccharose (10 to 20 per cent.), in ten to thirty minutes a crop of tiny colonies appears in the agar. The saccharose makes visible a process which is going on also without saccharose in the cultures.

In the bacterium cultures the amorphous substance forms capsules around the bacteria, and they are especially strongly developed around disintegrating bacteria. In certain stages of development the capsule of bacteria and also the halo seem to consist of fine filaments similar to bacterial flagella.

All these different observations suggest that the amorphous substance contains living elements different from the bacteria, although a definite proof of this supposition could not be obtained. The observations point to a new phenomenon of bacterial growth closely connected with capsule production.

*Prof. A. Fontes (Rio de Janeiro) : *Life-cycles of Bacteria. Symbiotic Associations. Filterable Forms.*

Starting from the study of the colon Bacillus, I give the following summary :—

(1) genesis of the colony ;
(2) formation of the bacilli ;
(3) reproduction or multiplication of the bacilli.

The lysis of the germ gives rise to the formation of the colony.

After the lysis the bacillary chromidium spreads, either growing at the surface of solid media, or flocculating and growing in suspension in liquid media.

There appear sometimes microcrystals, which serve the future colony for nucleus.

The granular aspect of the chromidium becomes afterwards more accentuated, and an outline of trabecular formations is seen.

The cycle of growth and multiplication of the bacilli is still based on the work of granular organization.

The bacterial chromidium differentiates itself in polar condensations, forming thus two new units by transverse division, or dissolves itself, giving birth to granular dust, the granules of which grow and give rise, in their turn, to new bacterial units or microcolonies.

The resolution of the chromidium into very fine granulations is to be considered as the first morphic phase of life within the borders of microscopical visibility.

The symbiotic associations, narrowly connected with the lytic phenomenon, appear after the granular organisation, due to the unequal distribution of the chromidial substance in certain elements, which will later on build new bacteria.

Due to this unequal distribution there arise several hereditary characters of the germs, formed by the loss or by the acquisition of biochemical properties.

Connected with these variations, the filterable elements appear due to the bacillary disintegration and correspond to infinitesimal particles of living substance, comprised within the limits of microscopical visibility.

These elements by taking their origin from the phase of granular resolution, behave as microbic variants having properties different from those of the primitive stem. The regenerative capacity of the barely viable elements may be recognised *in vivo* after some passages, which favour further cultures *in vitro*.

The pathogenic action of these elements is evidenced by peculiar characteristics (particular case of *B. tuberculosis*).

Prof. W. Schwartz (Karlsruhe): *Die physiologischen Grundlagen der Symbiosen von Tieren mit Pilzen und Bakterien.*

Symbiosen von Tieren mit Pilzen und Bakterien finden wir besonders bei Insekten. Wir müssen annehmen dass auch diese Gruppe von Symbiosen ähnlich wie z.B. die Mycorhizen, aus parasitären Infektionen hervorgegangen ist. Die genaue Untersuchung einzelner Symbiose-Fälle zeigt, dass auch heute noch parasitäre Störungen auftreten (Lecaniinen, Cikaden, Cimiciden, u.s.w.). Der Wirtsorganismus beeinflusst die Zahl und in den meisten Fällen auch die Gestalt seiner Symbionten. Dies wird deutlich, wenn man das Verhalten des Symbionten im Wirt und in der Reinkultur vergleicht oder wenn durch physiologische Veränderungen im Wirt, Gestalt und Vermehrungsrate der Symbionten geändert werden. Die Ursache für die qualitative und quantitative Beeinflussung der Symbionten liegt vermutlich in den Ernährungsverhältnissen während der Symbiose (Art und Menge der frei gegebenen Nahrung), ferner in spezifischen Abwehrreaktionen des Wirtes. Phagocytose ist in verschiedenen Fällen nachgewiesen worden. Eine grössere Bedeutung hat wahrscheinlich die humorale Abwehr. Darauf deutet hin, dass sich bei künstlicher Infektion von Nicht-Symbiontenträgern an den injizierten Bakterien dieselben Formveränderungen abspielen, die wir häufig auch in der Symbiose an symbiontischen Bakterien beobachten. Eine Symbiose im Sinne Buchners kann nur dann enstehen, wenn Kontaktmöglichkeit mit solchen Pilzen und Bakterien vorhanden ist, die sich dem betreffenden Wirt gegenüber zwar aggressiv verhalten, aber nicht oder nur wenig virulent sind,—und wenn der Abwehrapparat des Wirtes auf diese Art von Mikroorganismen nur unvollkommen reagiert. Die Symbiosen erscheinen also zunächst als ein abgeschwächter Parasitismus der Symbionten im Wirt; wenn sie sekundär für den Wirt lebensnotwendig werden, so ist dies nur möglich durch eine Umkehr des Parasitismus: der Wirt parasitiert jetzt auf seinen Symbionten und zeigt echte Ausfallerscheinungen, wenn man die Symbionten experimentell entfernt. Der Nachweis der Unentbehrlichkeit ist bei intrazellularen zyklischen Symbiosen allerdings schwer zu erbringen, er ist vielleicht Achsner bei Pediculus und Koch bei Anobium gelungen. Worin in beiden Fällen die Bedeutung der Symbiose liegt, wissen wir noch nicht. Selbst wenn hier die Symbionten nach Buchners Hypothese als Vitamin-Quelle dienen würden, so dürfte diese Feststellung keinesfalls verallgemeinert werden.

***Prof. C. Gorini** (Milan): *Les associations symbiotiques et les cycles évolutifs.*

(1) *Fermentation gazeuse associative*—Elle est caractérisée par l'aérogénie qui se manifeste dans un substrate sous l'action synergique de différentes bactéries qui isolément sont incapables d'y produire du gaz. Mes recherches sur ce phénomène dans le lait

donnent raison à Ishikawa selon lequel deux saccharolytes ne sont pas suffisants ; il faut en outre la présence d'un protéolyte. Même deux seules bactéries peuvent suffire, pourvu que l'une soit un saccharolyte gazéifiant sur le glucose (*B. morgani*), et l'autre un acidoprotéolyte, (*Streptococcus liquefaciens*).

(2) *Signification des differents microbes dans les symbioses*—Dans les procès symbiotiques les différents microbes ont importance non pas autant par leur nombre que par leurs activités biochimiques. Prenons l'exemple du procès de maturation fromagère, où travaillent en symbiose plusieurs bactéries, dont les principales sont les acidoprotéolytes, et les ferments lactiques. Les acidoprotéolytes pourvoient à la démolition de la caséine native, préparant la nourriture pour les ferments lactiques et poursuivant progressivement la caséolyse—jusqu'à l'affinage du fromage. Les ferments lactiques pourvoient à la démolition du lactose, donnant au caillé le degré d'acidité et d'égouttement nécessaire pour entraver les microbes dangereux du boursouflement, de la putréfaction, etc. Les acidoprotéolytes, surtout chez certains fromages et dans certaines phases de maturation, peuvent être rencontrés en proportion bien inférieure aux ferments lactiques. Cela a amené erronément plusieurs A.A. à les négliger et à donner importance exclusivement aux ferments lactiques, qui pourtant ne peuvent pas expliquer le procès de maturation, étant incapables d'attaquer la caséine native (Davis et Mattick) et de peptoniser.

(3) *Phases mobiles de vie.* La mobilité est une fonction bactérienne très inconstante, irrégulière. On attribue cette inconstance soit à la qualité physique ou chimique du substrate soit à la température d'incubation, etc. En 1903 j'ai proposé la méthode de la *culture grimpante* sur gélose inclinée. Par cette méthode j'ai démontré que plusieurs bactéries sont mobiles seulement dans leur phase juvénile. La mobilité serait une propriété des cellules qui sont dans un état particulier de germination, d'évolution du protoplasme.

***Prof. L. A. Silber** (Moscow) : *Symbiosis of Microbes and Viruses.*

(1) A number of different biological processes may occur in the case of a simultaneous presence of microbes and viruses in the same medium. These processes may be favourable to the preservation and multiplication of the virus or, on the contrary, may aid in its more speedy destruction.

(2) Some viruses are able to keep their viability for a much longer period in the presence of microbes than in a sterile medium.

(3) Adsorption of viruses by living microbes bears an elective character. Viruses adsorbed by microbes preserve their viability under such conditions under which without microbes they perish.

(4) Some viruses are able to multiply in microbial cultures. Micro-biologists in U.S.S.R. have cultivated on yeasts many viruses :

vaccinia, typhus, fowl pox, measles, encephalitis, encephalomyelitis, etc.

(5) Viruses grown in yeast cultures are changed in their biological properties. These alterations are observed both in regard to the character of the lesions provoked by them in the animals, and in that of their immunizing capacity, which is lowered.

(6) Yeast cells in such cultures lose their ability to adsorb electively certain stains from their mixtures with other stains. Simultaneously they suffer in their ability to eliminate stains and to reduce them.

(7) The successful obtaining of virus cultures on microbes depends to a high degree on the individual character of the strains of both virus and microbe and their suitable selection.

(8) Microbes may be carriers of viruses under natural conditions. Our experiments with vaccinia lymph demonstrate quite convincingly that the coccus flora of the lymph often contains vaccinia virus, while in the rod flora as a rule the virus is not to be found.

(9) The cases in which microbes are found to provoke virus diseases, not being, however, their actual causative agents, are explained by the phenomenon of virus carrying by microbes (allobiophoria).

(10) The relations indicated above do not, of course, exhaust all the possible forms of symbiosis between microbes and viruses.

(11) It is possible to utilize symbiotic phenomena for the cultivation of the filterable forms of some bacteria.

Dr. P. Hauduroy (Paris) : *Les formes invisibles des bactéries visibles.*

En 1910, Fontes avait affirmé l'existence des formes invisibles pour le bacille de Koch. En 1923, Vaudremer retrouve par une autre technique les faits avancés par Fontes, les amplifie considérablement et donne au problème, grâce à ses belles recherches, toute l'ampleur qu'il mérite. Le Professeur Bezançon et moi-même confirmons ces travaux et depuis lors, un nombre considérable de chercheurs sont consacrés à cette question.

Le premier, j'ai pu prouver l'existence des formes invisibles pour les *B. dysentériques*, les *B. typhiques* et *paratyphiques*, pour le *B. diphtérique*, pour les Streptocoques.

Depuis lors, de nombreux auteurs ont confirmé l'ensemble de mes recherches et ont pu, à leur tour, retrouver dans de nombreux cas, les formes filtrantes des bactéries non filtrantes. Toutes ces formes, de quelque bactérie qu'elles proviennent, se rapprochent par certains de leurs caractères des ultravirus ou infra microbes.

Mais elles ne sont pas des ultravirus.

On parle couramment, à l'heure actuelle, d'ultravirus tuberculeux, d'ultravirus typhique, pesteux etc.

Il n'y a pas d'ultravirus tuberculeux, Il y a des formes filtrantes du bacille tuberculeux ; *il n'y a pas d'ultravirus typhique*, il y a des formes filtrantes du bacille d'Eberth, etc.

L'ultravirus est un être bien déterminé, la forme filtrante d'une bactérie visible un autre être, bien déterminé aussi. Ils ont tous deux quelques caractères communs, mais ils ont aussi une majorité de caractères différents. Appelons-les tous deux " matières invisibles " si l'on veut.

La forme filtrante d'une bactérie n'est qu'une phase d'un cycle parcouru par cette bactérie. *Elle est capable, grâce à certaines de ses propriétés physiques (dimensions, charge électrique), de traverser certains filtres ou ultrafiltres que ne traverse pas la bactérie dont elle provient placée dans les mêmes conditions. La forme filtrante est invisible à un moment donné, peut présenter à d'autres moments une morphologie aberrante, alors que, en usant des mêmes procédés de coloration, la bactérie dont elle provient est visible. La forme filtrante est, habituellement, cultivable dans les milieux bactériologiques usuels. La forme filtrante possède, en général, un pouvoir pathogène différent de celui de la bactérie dont elle provient. La forme filtrante est produite par des mécanismes connus (bactériophage) et inconnus (bacille tuberculeux). Il est toujours possible, caractère fondamental, de reconstituer la bactérie à partir de la forme filtrante, fermant un cycle évolutif.*

On a violemment critiqué les techniques permettant d'obtenir ces formes et en particulier la filtration.

La filtration est à la fois la meilleure et la pire des techniques suivant qu'elle est bien ou mal employée.

Mais pour obtenir les formes invisibles de certains microbes visibles il n'est pas nécessaire de filtrer.

Lysons avec le bactériophage 100 c.c. d'une culture en bouillon de *B. coli*, par exemple. La lyse obtenue, *ne filtrons pas*, et répartissons le produit obtenu en 20 ampoules de 5 c.c. chacune. Vérifions par un séjour à l'étuve de 24 heures qu'aucune culture n'apparaît, que la lyse a été complète et qu'aucune faute n'a été commise. Laissons les 20 ampoules à la température du laboratoire et observons les pendant plusieurs mois. Nous verrons, *à peu près certainement*, apparaître après des délais variables, des troubles dans un certain nombre de ces ampoules. Ces troubles sont constitués par des éléments-granules, par une " substance non organisée " qui ne cultivent *normalement* sur aucun milieu connu. Il sera cependant possible, grâce à la technique que j'ai indiquée, de reconstituer des *B. coli* à partir de ces éléments.

Quel rôle jouent les formes invisibles des microbes visibles en pathologie humaine, animale ou végétale ?

Nous ne le savons pas encore, mais tout ce qui a été découvert, permet de construire des hypothèses pleines d'avenir.

On peut, sans être taxé d'imprudence, supposer que l'étiologie d'assez nombreuses maladies infectieuses sera prochainement à réviser et qu'on s'apercevra alors qu'elles sont dues non pas aux formes visibles des germes pathogènes, mais à leurs formes invisibles.

Dr. M. B. Soparkar (Parel, Bombay) : *The Filtrable Form of the Tubercle Bacillus.*

The author has conducted several experiments using autolysed sputum containing human bacilli and ground-up tissues of animals inoculated with virulent bovine bacilli for filtration and subsequent inoculation of the filtrate into guinea-pigs, later making successive passages of the material in series from guinea-pig to guinea-pig. The positive results obtained were based not merely on the finding of acid-fast bacilli in the inoculated guinea-pigs, but on isolation from them of pure cultures of acid-fast bacilli obtained in six out of ten experiments, and five of these strains were studied. Filtrates from three sputa containing human bacilli and two animal tissues containing bovine bacilli yielded through guinea-pigs pure strains which differed from the original strains and showed pathogenic characters resembling the avian bacillus, and in broth they produced homogeneous cultures. In two instances an intermediate stage was also found in which the strain was non-pathogenic to the guinea-pig, rabbit and the fowl. Similar results of filtration experiments yielding avian-like bacilli have been obtained by Valtis, Nègre and van Deinse.

Control experiments were designed to carry a large series of guinea-pigs through the same process of inoculation, passages, exposure to laboratory conditions, etc., as the previous batch of filtration experimental guinea-pigs, the only difference between the two batches being that the control guinea-pigs were not inoculated with any filtrate. Twelve such experiments were made with 9 passages in each experiment involving the total use of 117 guinea-pigs. The total exposure of these animals to laboratory conditions extended to 315 days, as compared to only 263 days in the case of the filtration experiments. Cultures were made on 65 occasions from tissues of several suspected guinea-pigs, but in not a single case was a culture of acid-fast bacilli obtained, while in the original filtration experiments 91 cultural experiments had yielded pure growths of acid-fast bacilli on as many as 22 occasions. These entirely negative results of control experiments left no doubt that the positive results obtained in the previous filtration experiments could not have been due to any spontaneous infection of these guinea-pigs.

These results point to the presence of a filtrable form of the tubercle bacillus which can be recovered from inoculated guinea-pigs and yields pure cultures of acid-fast bacilli. These bacilli differ from the original strains and present varying biological characters and often resemble the avian tubercle bacillus.

The experiments of Valtis, Nègre and van Deinse have further shown that these avian-like bacilli can again be transformed into classical human or bovine tubercle bacilli. This observation further supports the view that these irregular avian-like strains of acid-fast bacilli represent the filtrable form of the tubercle bacillus.

Dr. Emmy Klieneberger (London) : *On a New Group of Microbes occuring as Symbionts of Bacteria and independently.*

In my studies of the *Streptobacillus moniliformis* all examined cultures were shown to contain, besides the streptobacilli, a second organism showing no bacillary elements. It is composed of filterable granules, filamentous structures and globules similar to those of the organisms of pleuropneumonia and agalactia. This second symbiotic organism was for convenience designated L_1, and its isolation in pure culture can be achieved by three different methods. The perpetual culture of these pure L_1 strains is possible on special media.

The L_1 organism occurs naturally not only as a symbiont but also independently. To all investigators using laboratory rats a condition of their lungs is known which is characterized in the early stage by a number of discrete, cyst-like foci on the pleural surface and develops into a bronchiectasis ; the cavities contain first a gelatinous, later a glairy, viscous substance. Hitherto no organism has been found regularly occurring in these lungs, but recently I was able to grow regularly from these lesions numerous colonies of the L_1 organism on my special medium. The strains obtained from the lungs show the same cultural and morphological characteristics as the L_1 strains originating from the symbiotic streptobacillus cultures. It has not yet been possible to study the relation between the L_1 organism and this lung disease of the laboratory rat in infection experiments. However, two interesting facts throw some light on the problem : (1) the constant presence of the L_1 organism in the lesions, (2) the frequent occurrence of the *Streptobacillus moniliformis* in the nasopharynx of the infected rats.

Apparently *Streptobacillus moniliformis* is not the only case of a symbiosis between a bacterium and a pleuropneumonia-like organism. In a number of publications Theobald Smith described cultures, isolated from the lung lesions of calves which had suffered from an infectious bronchopneumonia. This organism, which he designated *B. actinoides*, closely resembles *Streptobacillus moniliformis* and represents apparently another symbiotic association of this type.

According to my last investigations M. Knorr's *Fusobacterium nucleatum* might be another example of an association of two different organisms. I suppose that a few more cultures in which peculiar forms occur will one day be revealed as symbiotic associations ; but the morphological demonstration is not sufficient, cultivation methods ought to be improved so that separation and independent culture of the symbionts may be achieved and their serological and pathological reactions may be studied.

Prof. E. M. Fraenkel (London).

In efforts to decide the question of the existence of a filtrable virus of tuberculosis we have, so far, examined 8 strains of tubercle bacilli in 60 experiments. 7 different strains of human bacilli have been examined in 35 experiments and the bovine strain

" Vallée " occupied 25 experiments. In these experiments we have used Chamberland L2 candles and standardized Bechold collodion membrane filters, the size of the pores being 0·75μ, 0·3μ and 0·1μ.

As recent publications by Plotz and others have shown that there exist differences in the electric charge and the consequent filtrability of the " S " and " R " types we have paid particular attention to the presence of dysgonic " S " forms in our strains. Experiments with the strain " Bovine Vallée," where " R " and " S " forms were both present, gave a total of 8 positive results in 25 experiments.

Of the 7 human strains 3 were found to be filtrable, giving 6 positive results in a total of 17 experiments. The remaining 4 strains gave completely negative results in 18 experiments. I emphasize the point that in the case of the filtrable strains 2 out of 3 contained both " R " and " S " types, whereas in the case of the non-filtrable strains only one showed the presence of an " S " type. It is perhaps more impressive to show that 7 experiments with the strain " P.1." (a consistently " R " type) gave completely negative results, whereas the strain " Hiscock " gave 4 positive results in 7 experiments and was invariably observed to contain both " S " and " R " types.

Cultural examinations were carried out on Sauton broth and on Löwenstein-Jensen medium.

Histological examination by Dr. R. J. V. Pulvertaft showed that tubercular lesions were invariably obtained in those guinea-pigs injected with filtrates considered to be positive. These lesions were either completely localized or there existed a few foci in the spleen or liver. Tubercle bacilli were found either in the lesions themselves or upon transplantation to another guinea-pig, where generalized tuberculosis always resulted. Bacilli were never found, however, in the filtrates which were examined both microscopically and by cultures.

Filtrability and the Occurence of " R " and " S " Types.

Strain	" R " Type	" S " Type	Filtrability (No. of positive Expts.)
P. 1	+	—	Nil
Dainlow	+	+	Nil
Ratti " R " ...	+	—	Nil
Bishop	+	—	Nil
Hemmings ...	+	—	1 out of 5
34/31	+	+	1 out of 5
Hiscock	+	+	4 out of 7
Bovine Vallée ...	+	+	8 out of 25

Dr. G. Seiffert (München), **Dr. M. Gutstein** (London), and **Dr. A. Grumbach** (Zürich), also took part in the Discussion.

MICELLANEOUS PAPERS

Chairman : **Prof. A. Fleming** (London).

Prof. J. von Darányi (Budapest) : *Nachweis der Pathogenitatät der Staphylokokken.*

Wegen des ubiquitären Vorkommens von Staphylokokken können leicht " Umgebungsstaphylokokken " in bakteriologische Untersuchungsmaterialien hineingeraten. (Vgl. meine früheren Mitteilungen).[1,2] Es ist deshalb notwendig mit Staphylokokken aus Umtersuchungsmaterialien eine " Pathogenitätsprüfung " vorzunehmen. Die pathogenen Staphylokokken zeichnen sich vor den apathogenen durch stärkere Fermentbildung aus, die zum Ausdruck kommt in Gelatineverflüssigung, Milchgerinnung (ev. Verflüssigung des Kaseinkoagulums), Haemolyse, Fibrinogengerinnung und Nekrose- Eiter- Erregung im Tierversuch. Gelatineverflüssigung und Milchgerinnung sind nicht konstant und können auch bei pathogenen Arten fehlen. Für die Diagnose sind in erster Linie Haemolyse, Fibrinogengerinnung und Nekrose- Eiter- Erregung zu verwenden.

Die pathogenen Staphylokokken zeigen immer Haemolyse. Doch auch saprophytische Staphylokokken können haemolysieren, wenn auch meist nur auf 5—10 prozentigen Hammelblutagarplatten. Pathogene Stämme haemolysieren 20—40 prozentige Hammelblutagarplatten. Deshalb soll zur Prüfung der Haemolyse ein dünner Ausstrich auf 30 prozentigen, frisch bereiteten Hammelblutagarplatten angelegt werden. Die Besichtigung soll bei Tageslicht geschehen.

Noch zuverlässiger ist der Nachweis der Fibrinogengerinnung, die ich 1926 als erster für pathogene Staphylokokken beschrieben habe. Zur Prüfung wird 2 prozentige Natriumcitrat- Kochsalzlösung zu gleichen Teilen mit Kaninchenherzblut vermischt. In 1 ccm dieser Mischung wird 1 Oese Agarkultur langsam (zunächst an der Wand des engen Röhrchens) verrieben. Pathogene Arten rufen bei 37 Grad C in einigen Stunden Gerinnung hervor. Diese spezifische Koagulation pathogener Staphylokokken ist gelatineartig; Kondenswasser wird nicht ausgepresst. Staphylokokkentoxin (filtrierte Bouillonkultur) erzeugt diese Gerinnung nicht.

Zur Prüfung auf Nekrose- Eiter- Erregung wird 1 Oese der auf Löfflerserumnährboden gewachsenen Kultur an der inneren Fläche des Hinterschenkels des Kaninchens in eine 1—2 ccm tiefe Hauttasche eingeführt. Diese Methode ist der von Dold angegebenen überlegen. In 2—3 Tagen entsteht eine weissgelbe nekrotische Fläche, unter der sich eine eiternde Fistel befindet.

Von den charakteristischen Merkmalen der pathogenen Staphylokokken ist nach meinen Versuchen die Nekrose- Eiter- Erregung (Virulenz) die am schnellsten verschwindende Eigenschaft. Die

[1] *Zbl. Bakt.*, Orig. I., 1926, 99, 74 ;
[2] *Wien. Med. Wochenschr.*, 1927, Nr. 5.

Fibrinogengerinnung bleibt länger erhalten und am längsten die Haemolyse. Durch zweimalige Mauspassage (Rückenhaut, Abimpfung von der Maus nach 2 Tagen) gelang mir neuerdings eine Haemolysesteigerung eines nur schwach haemolysierenden Stammes.

Zusammenfassung.

(1) Pathogene Staphylokokken zeigen noch auf 20—40 prozentigen, defibrinierten Hammelblutagarplatten Haemolyse.

(2) Pathogene Staphylokokken bewirken eine gelatineartige Koagulation des Citratblutes. Diese Fibrinogengerinnung ist das sicherste Kennzeichen der Pathogenität, da sie niemals von nichtpathogenen Kokken erzeugt wird. Auch andere Bakterien rufen diese Erscheinung nie hervor.

(3) Im Kaninchenversuch erzeugen pathogene Staphylokokken, subcutan eingebracht, Nekrose und Eiterung. Frischisolierte Kulturen rufen diese Erscheinung regelmässig hervor, vorausgesetzt dass sie auf Löfflerserumnährboden gezüchtet wurden.

(4) Die haemolysierende Fähigkeit der Staphylokokken kann durch Mauspassage erhöht werden.

Prof. J. Hirsch (Istanbul): *Zur Bestimmung der Vermehrungsgeschwindigkeit aerober Bakterien.*

Bei der Untersuchung von bakteriellen Stoffwechsel- und Fermentleistungen mittels der gasanalytischen Mikromethode von Otto Warburg werden die beobachteten Umsetzungen (z.B. der Sauerstoffverbrauch in bestimmten Substraten) auf eine *konstante* Masse lebender Substanz bezogen; d.h. auf eine konstante Zahl von Bakterien. Zu diesem Zweck verwendet man Bakteriensuspensionen in Lösungen, in denen eine Vermehrung der Bakterien ausgeschlossen ist, die vor allem frei von assimilierbaren Stickstoffsubstanzen sind. Man beobachtet also bestimmte biochemische Leistungen *nicht* in Vermehrung begriffener, "ruhender" Bakterien.

Es wird die Frage zur Diskussion gestellt, inwieweit es möglich ist, die manometrische Mikromethode von Warburg für das Studium der *Vermehrung aerober Mikroorganismen* nutzbar zu machen.

Züchtet man aerobe Bakterien in den Reaktionsgefässen der Barcroft-Warburg-Apparatur in einem zur Keimvermehrung geeigneten Milieu (z.B. in Bouillon), so zeigt die manometrische Bestimmung des Sauerstoffverbrauches, dass die Atmungsgrösse pro Zeiteinheit mit der Zunahme der lebenden Substanz—d.h. mit der Vermehrung der Bakterien ansteigt.

Während die Kurve der Atmungsgrösse "ruhender" Bakterien parallel zur Abszisse der Zeit verläuft, hebt sich die Kurve der Atmungsgrösse einer in Vermehrung begriffenen Kultur steil nach oben.

Gleichzeitige Bestimmungen der jeweils vorhandenen Zahl der lebenden Keime und der jeweiligen Atmungsgrösse ergeben für die Hauptphase der maximalen und konstanten Vermehrungsge-

schwindigkeit (Buchanan's "logarithmic growth phase"), dass die Verdopplung der Atmungsgrösse etwa die gleiche Minutenzahl erfordert wie die Verdopplung der Keimzahl.

In der halblogarithmischen graphischen Darstellung erscheinen die Kurve der Keimzahl und die Kurve der Atmungsgrösse als grade Linien mit gleicher Neigung zur Abszisse der Zeit.

Innerhalb der Hauptphase der Vermehrung ist die Geschwindigkeit der Atmungssteigerung ein quantitativer Ausdruck für die Geschwindigkeit der Keimvermehrung. Hieraus ergibt sich eine brauchbare Methode zur Bestimmung der Vermehrungsgeschwindigkeit aerober Bakterien.

In Kulturen aerober Bakterien mit verschiedener Einsaat (z.B. mit Einsaaten zwischen 10^7 und 10^2 Keimen pro cm^3 Kultur) verläuft die Steigerung der Atmungsgrösse—innerhalb der "logarithmic growth phase"—mit der gleichen Geschwindigkeit. In der halblogarithmischen graphischen Darstellung erscheinen die Kurven der Atmungsgrössen der verschiedenen dicht beimpften Kulturen—innerhalb der Hauptphase der Vermehrung—als parallel gerichtete gerade Linien. Ihr Abstand von einander (gemessen an der Abszisse der Zeit) ist abhängig von der Abstufung der Einsaat; im Falle einer gleichmässigen Abstufung ist der Abstand der Parallelen untereinander gleich. Hieraus ergibt sich eine weitere Versuchsanordnung zur Bestimmung der Vermehrungsgeschwindigkeit aerober Bakterien, in der alle Ungenauigkeiten einer Keimzählung vermieden werden und in der lediglich eine exakt durchgeführte Verdünnung der Einsaat erforderlich ist.

Man beschickt je ein Reaktionsgefäss (oder mehrere) mit gleichen Mengen einer Nährlösung, die mit einer beliebigen Zahl von Keimen pro cm^3 (*a*) und mit einer bestimmten Verdünnung dieser Keimzahl ($\frac{a}{n}$) beimpft sind. Man stellt fest, wie gross der Zeitunterschied (T) ist, um den die Kultur mit der Einsaat $\frac{a}{n}$ später als die Kultur mit der Einsaat a eine bestimmte Atmungsgrösse innerhalb der "logarithmic growth phase" (z.B. die Atmungsgrösse 50 $mm.^3$ O_2 in 15 Minuten pro $cm.^3$ Kultur) erreicht. Diese Zeitspanne (T.) multipliziert mit dem Faktor $\frac{\log 2}{\log n}$ ergibt die durchschnittliche Generationsdauer der betreffenden Mikroorganismen unter den gegebenen Versuchsbedingungen.

Die beschriebenen Verfahren eignen sich für die experimentelle Bearbeitung der verschiedensten theoretischen und praktischen Probleme (z.B. zur Untersuchung des Einflusses der Temperatur, der verschiedenen Brennstoffe, Baustoffe und Wuchstoffe auf die Vermehrung aerober Mikroorganismen; zur Charakterisierung der Vermehrungsgeschwindigkeit verschiedener Erreger unter gleichen Bedingungen; zur Prüfung der entwicklungshemmenden Wirkung von Desinfektionsmitteln und dergleichen mehr).

(Ausführliche Darstellung der Methoden: Revue de la Faculté des Sciences de l'Université d'Istanbul. Tome I, Fasc. 3, pag. 44–60, Avril, 1936).

Dr. J. G. Davis (Shinfield, Reading): *The Metabolic Method for the Identification of Bacterial Species.*

The biochemical methods used for identifying bacteria consist very largely of growing them in various media and detecting the end products of metabolism. Saccharolytic bacteria are identified largely by their ability to produce acid from certain sugars, acetyl methyl carbinol from dextrose, etc. A common cause of discrepancies between laboratories is the different conditions under which these tests are made. Further, these tests involve the growth and "dying out" of bacteria—*i.e.*, cells in all three phases of growth are present. Changes may be due to exogenous, endogenous or both types of enzymes. A considerable time elapses before all the tests are completed.

A metabolic investigation in the Warburg-Barcroft apparatus necessitates only a massive pure culture in a liquid medium. This is examined for purity, and aseptic precautions are then no longer required. The bacteria are centrifuged off, washed, and emulsified in suitable buffer solutions. Manometric measurements in the Warburg vessels are made for 1–2 hours and micro-chemical analyses performed on the final suspensions. Various aspects of metabolism may thus be studied quantitatively.

In effect, the "metabolic method" provides a quick, more rigorously standardized and reproducible method of carrying out most of the "biochemical" tests in use in bacteriological laboratories. The many factors causing variations in the results obtained in different laboratories are reduced to one—the previous life history of the micro-organism, the nature of the culture medium used in this technique being the most significant factor in this.

The method is of particular use in differentiating bacteria which are superficially alike—*e.g.*, the normal and a- varieties of *L. delbrueckii.*

[A demonstration of the apparatus and method was given at the National Institute for Research in Dairying on Friday, 31st July, 1936.]

Dr. A.v. Mallinckrodt-Haupt (Düsseldorf): *Die Porphyrinbildung durch Mikroorganismen.*

Im Anschluss an Untersuchungen bei Hautsaprophyten, die zur Klärung der Hautfluoreszenz vorgenommen wurden und eine *überraschend hohe Anzahl porphyrinbildener Keime* ergeben hatten, wurden systematische Untersuchungen der verschiedenen Mikroorganismenarten auf die Bildung von Porphyrinen und von anderen fluoreszierenden Substanzen vorgenommen.

Die Befunde bei etwa 70 von menschlicher Haut gezüchteten *Schimmelpilz*-Kulturen waren negativ. Ebenso hatten frühere Untersuchungen ergeben, dass die pathogenen Hautpilze—Favus, Mikrosporon, Trichophyton, Epidermophyton—kein Porphyrin produzieren.[8]

Bei den vorliegenden Versuchen an 30 wilden Hefenstämmen, die grösstenteils von gesunder menschlicher Haut gezüchtet waren, fand ich nur 5 Porphyrinbildner. Ausserdem wurden 3 sicher pathogene Stämme untersucht: *Oidium asteroides* Cast., *Monilia tropicalis* u. *Monilia Guilliermondi*, die alle 3 positive Resultate ergaben.

Auffallend ist das Verhalten der *Streptothricheen*: alle untersuchten Stämme (22) zeigten *ausserordentlich starke Porphyrinbildung*, sodass es sich hier, abgesehen von allen anderen Fragestellungen, auch um ein *diagnostisch verwertbares Merkmal* für diese Gruppe zu handeln scheint. Bei der grossen Bedeutung, die gerade die Streptothricheen unter den Bodenmikroorganismen besitzen—sie bilden bekanntlich etwa 30% aller vorkommenden Erdkeime—dürfte vielleicht dem von ihnen gebildeten Porphyrin eine Rolle als *Lichtsensibilisator bei der Humusbereitung* zukommen. Wir wissen ja, dass die Humusbereitung in Abhängigkeit vom Sonnenlicht steht u. dass im Schatten der unerwünschte saure Humus gebildet wird. Versuche in dieser Richtung sind im Gang.

Unter den seinerzeit von der menschlichen Haut gezüchteten *saprophytischen Stäbchen* (73 Stämme) waren *ein Drittel Porphyrinbildner*.

Von diesen Befunden ausgehend, wurden eine Reihe der bekanntesten Bakterienarten, besonders auch tier- u. menschen*pathogene* Stämme in gleicher Weise auf Porphyrinbildung untersucht, doch *mit vollständig negativem Erfolg*.

Die Untersuchung erstreckte sich auf 59 Kulturen: Abortus Bang (20), Lactis aerogenes (1), Pneumoniæ Friedländer (1), Paratyphus A (1), Paratyphus B (1), Enteritidis Gärtner (1), Bresloviense (1), Suipestifer (1), Voldagsen (1), Abortus equi (7), Typhus pullorum (4), Prodigiosum (2), Pyocyaneum (1), Proteus (3), Erysipelatos suum (3), Coli mit u. ohne Indolbildung (8), Fæcalis alcaligenes (1), Dysenteriæ Flexner (1), Subtilis (1).

Unter sämmtlichen *Kokkenarten* (44 Stämme)—in der Hauptsache wurden Staphylokokken, aber auch Strepto- und Pneumokokken untersucht—fand sich kein Porphyrinbildner. Nur ein *Enterokokkus* zeigte geringe Porphyrinmengen.

Was die *Natur der gefundenen Bakterienporphyrine* anbelangt, so ergab die Untersuchung mit dem Gitter-Mess-Spektroskop nach Löwe-Schumm und Vergleich mit reiner Kopro-bezw. Protoporphyrinlösung, dass es sich bei *Streptothricheen* und *Stäbchen* um *Koproporphyrin* handelt.

Ich fasse nocheinmal kurz zusammen: Während die banalen und pathogenen Kokken, soweit sie bisher untersucht wurden,

bis auf einen Enterokokkus keine Porphyrinbildung zeigen, sind die Befunde bei Streptothricheen zu 100% positiv. Die Versuche bei Schimmelpilzen verliefen negativ. Die Beteiligung der Hefen beträgt etwa 24%, darunter finden sich auch hautpathogene Stämme. Auffallend ist der hohe Prozentsatz von Porphyrinbildnern unter den hautsaprophytischen Stäbchen, während die Untersuchung einer Reihe von Stäbchen, die an der Zusammensetzung der Bakterienflora des Darmes beteiligt sind, sowie anderer tier- und menschenpathogener Bakterien vollständig negativ verlief. *Die Porphyrinbildung durch Mikroorganismen ist also keineswegs selten, doch scheint sie auf bestimmte Gruppen beschränkt zu sein. Unter den Saprophyten ist sie häufiger als unter den pathogenen Arten.*

Die Aufklärung der Zusammenhänge der Porphyrinbildung mit medizinischen und anderen biologischen Problemen—insbesondere mit einer eventuellen *lichtsensibilisierenden Wirkung*—wird das Ziel weiterer Forschung sein.

Dr. C. Rimington (Onderstepoort, South Africa) in discussing Dr. Mallinkrodt-Haupt's communication, said that the disease "geeldikkop" affecting sheep in South Africa was caused by the ingestion of the plant *Tribulus spp,* (*Zygophyllaceæ*) and was characterized by photosensitization accompanied by an intense obstructive icterus. The photo-activating pigment was isolated from the blood and bile of geeldikkop cases and found to be identical with the chlorophyll derivative *phylloerythrin.* Even in the normal herbivorous animal, this porphyrin was present in the fore and true stomachs, small intestine and colon, but was excreted by the healthy liver into the bile.

Quantitative investigation showed that the principal seat of phylloerythrin formation in the sheep was the rumen and it was in this organ that the greatest number of symbiotic micro-organisms were found—yeasts, protozoa and bacteria. Unfortunately, no method was known by which these organisms might be cultivated outside the animal body, so that pure cultures could not be studied. It was found, however, that chlorophyll added to broth cultures of *B. coli* was degraded, in part, to a mixture of pigments including phylloerythrin.

That bacteria play only a minor rôle in the intravital production of phylloerythrin and that the yeasts and protozoa of the alimentary tract were the agents chiefly responsible was demonstrated by a quantitative comparison of the phylloerythrin content and microbial flora of samples of ingesta taken at various levels of the alimentary canal in the following species: sheep, calf, horse, rabbit, guinea-pig on mixed diet and pig, rat, dog and fowl after receiving chlorophyll by stomach-tube. Phylloerythrin was most abundant in those localities where symbiotic protozoa were most numerous.

Mr. N. W. Barritt (Rothamsted, England): *Concentration of Nutrient as a Dominant Ecological Factor in Mixed Colonies of Bacteria, Fungi and Protozoa.*

Mixed colonies of bacteria, fungi and protozoa occur in a more or less stable state of equilibrium in soil and in activated sludge used in sewage purification.

When such a colony is inoculated into a sterile culture solution, the equilibrium may be disturbed so that one or other of these groups of organisms becomes dominant. At a concentration above 0·05 per cent. peptone or sugar and ammonium phosphate no growth of protozoa occurs, but fungal growth predominates at concentrations of 0·2 per cent. and above. No fungal growth occurs at concentrations below 0·1 per cent.

Bacterial growth occurs at all concentrations, but is more dispersed at higher concentrations and is more agglutinated or in the form zooglœa at low concentrations.

These observations have an important bearing on the biological processes in soil and activated sludge. They also illustrate the difficulties of obtaining accurate counts of protozoa, by the plate method.

The enormous differences in protozoan counts obtained by the use of the standard peptone-lemco and of soil extract can be largely accounted for by the difference in concentrations of the nutrients used.

Isolation of bacteria from soil, using the nutrient media commonly employed in medical bacteriology, has shown that species capable of producing gas from the sugars are remarkable few, a fact which is hardly consistent with the rate of respiration characteristic of soil.

Also, protozoan counts by the dilution method using the standard peptone medium have shown a remarkable similarity in soils from all parts of the world and a preponderance of flagellates. Perey, Dixon, and others have found a much greater variety of species more especially of ciliates, when the cultures are grown on soil extract or hay infusion media.

Activated sludge contains many types of soil fungi, bacteria and protozoa. When inoculated into culture media of different concentrations it has been found that very different pictures of the flora and fauna are obtained. The following list shows the nature of the organisms predominating at different concentrations:—

Strength of medium as oxygen uptake in parts per 100,000.	200	150	100	50	0
Dominant organisms	Fungi, yeasts	Actinomycetes	Bacteria, Flagellates, Amœbæ (Threads, rods, spirochætes)	Ciliates, (Paramœcium, Colpoda, Stylonychia, Chilodon, Gastrostyla) Bacterial Zooglœa	Ciliates, (Aspidisca, Epistylis, Vorticella, Carchesium) Rotifers

It has been found that the nature of activated sludge varies more with the strength of the sewage treated than with its composition.

Dr. I. H. Maclean and **Dr. E. W. Fish** (London): *Streptococcal Cultures from Normal Tissues.*

In a series of 200 normal teeth removed *in situ* at post-mortem none showed any organisms in the pulp chamber or around the apex of the tooth, yet in 50 teeth extracted by the best surgical skill from clean healthy mouths 44 showed positive cultures of streptococci from the apex and from the pulp, and in all those examined in serial section, streptococci could be seen in the pulp tissues. Around these organisms in the pulp there was no cellular reaction so, presumably, they were not there during life. The only teeth which gave sterile cultures from the apex and pulp, and showed no organisms in section, were those in which the pocket around the neck of the tooth had been sterilized by a cautery before extraction.

Okell and Elliot showed that there was a transient bacteræmia following multiple extractions in cases of pyorrhœa. Our observations confirmed this, but we have shown that in such cases bacteræmia could be avoided if the pocket is cauterized before extraction. We concluded that (*a*) in spite of histological evidence to the contrary the pulps and apices of healthy teeth are sterile, (*b*) granulomata, except on extracted teeth, are sterile and are merely collections of small round cells in response to the infiltration of toxic products of bacteria, and (*c*) when infected, the cellular reaction becomes that of a true abscess with polymorphs and necrotic material constantly present.

Dr. J. Drbohlav (Prague): *The Problem of Cultivation of Sensitive Organisms out of Material received from considerable Distances, regarded from the Epidemiological Point of View.*

The principles of heating and cooling mixtures have been studied in order to solve the problem of an automatic micro-incubator for dispatching infectious material of bacteriological or parasitological origin. The Dewar vessel itself is not sufficient for maintaining the necessary temperature for a prolonged time. A special heating element filled with a substance maintaining the given temperature has been adopted. The method is based on the principle of thermodynamics and the substances chosen must have suitable points as regards: (*a*) solidification; (*b*) reversing; (*c*) eutectic activity. In final practical experiments extending over a period of two years an apparatus called " Monotherm " suggested by Prof. Mislowitzer of Beograd, has given satisfactory results. The heating element contains a mixture of aliphatic acids of melting point of 37·3°C. Its function is characterized by exploiting the heat of crystallization of the mixture activated by submerging the element into the boiling

water. The Dewar vessel into which the heating element is inserted works as insulating cover, increasing its thermal capacity. The shaking of the apparatus during the transportation increases the duration of the thermal arrest of the mixture and it helps to keep the given temperature much longer. The use of " Monotherm " reduces practically to nothing the first phase of the loss of time in present bacteriological methods. Material sensitive to cold, *e.g.*, meningococci, give a positive culture in cases where the present way of dispatching such material failed. The device is of inestimable value when carrying out epidemiological studies, in bacteriology and parasitology. The function of the apparatus is simple and does not involve any cost of maintenance.

Dr. Luis E. Hurtado (Guatemala, Central America): *Erythrococcus in Blood.*

In a small town (average temperature 20° C.) of 50,000 inhabitants, Comayaguela, Honduras, Central America, the drinking water flows through mineral beds of gold and silver. In spite of little attention to the hygienic disinfection of the water cases of intestinal infections caused by typhoid and paratyphoid bacilli are very rare. This is probably due to the mineral constitution of the ground. But fever lasting ten to fifteen days frequently occurs.

The onset is marked by angina, bronchial symptoms, cough and constipation. The fever is of the remittent type, descending by lysis in three or five days accompanied by diaphoresis.

The important thing is that blood culture in bouillon neutral to litmus produces after six or eight days at 37° C. a coccus smaller in size than staphylococcus; viewed microscopically it is yellowish red, but red when seen macroscopically, forming a thick sediment in the bottom of the flask. The coccus is Gram-negative and the culture on bouillon is agglutinated when mixed with the serum of convalescents.

The malady, which is not grave, attacks both sexes—especially the young. My point is to call attention to this chromococcus which produces a typical septicæmic infection with predominant bronchial symptoms.

A complete bacteriological study of this germ has not been worked out because adequate facilities are not available in this small isolated town.

SECTION 2

VIRUSES AND VIRUS DISEASES IN ANIMALS AND PLANTS

DISCUSSION *on* THE GENERAL CHARACTERISTICS OF VIRUSES, INCLUDING BACTERIOPHAGE

Chairman : Prof. R. Doerr (Basel).

Prof. R. Doerr (Basel), **Opener.**

Eine streng wissenschaftliche und in jedem einzelnen Falle anwendbare Definition des Virusbegriffes wäre nur dann möglich, wenn biologische Kriterien existieren würden, welche allen " Virusarten " gemeinsam sind und welche gleichzeitig nur diese Kategorie von übertragbaren Infektionsstoffen auszeichnen. Bisher konnte aber keine Eigenschaft ermittelt werden, welche diesen beiden Forderungen restlos genügen würde. Diese Sachlage wird auch in Zukunft keine Aenderung erfahren. Mit der Zunahme der tatsächlichen Kenntnisse wird es nämlich in steigendem Grade klarer, dass der Austruck " Virus " eine biologische Mannigfaltigkeit umspannt, die auch dadurch nicht auf eine Einheit rednziert werden kann, dass man einzelne Gruppen wie z.B. die Erreger der Pleuropneumonie und der Agalaktie, die pathogenen Agenzien der Hühnertumoren oder die Bakteriophagen rein willkürlich, d.h. mit beliebig wechselnder Begründung ausscheidet. Einheitlich ist das Objekt der Virusforschung lediglich durch die für seine Untersuchung brauchbaren Methoden und selbst in dieser Beziehung ist es vorwiegend negativ gekennzeichnet.

Die Aussage, dass die Elemente sämtlicher Virusarten einem bestimmten Grössenbereich angehören und dass die Methodik eben dadurch bestimmt wird, ist zwar an sich richtig, stellt aber kein gemeinsames biologisches Merkmal fest. Die Teilchen der Variola-Vaccine oder der Psittacose sind (dem Volumen nach) ca. 15-20.000 mal grösser als jene des Virus der Maul-und Klauenseuche und innerhalb dieser so weit auseinanderliegenden Grenzen lässt sich keine Abhängigkeit irgendeiner vitalen Eigenschaft von der Dimensionierung konstatieren. Es gibt mit andern Worten keinen oberen Grenzwert der Partikeldurchmesser für die Bildung von Einschlusskörperchen, für die Unfähigkeit der Vermehrung auf unbelebten Nährmedien, für die verschiedenen Antigenfunktionen, für hochspezifizierte Organotropie oder für eine ungewöhnliche Resistenz gegen äussere Einflüsse—vorausgesetzt, dass die Ergebnisse der Messungen als hinreichend genau anzusehen sind.

Unter der gleichen Voraussetzung muss hervorgehoben werden, dass mit der abnehmenden Teilchengrösse keine Convergenz bezw. Summierung jener Eigenschaften und Phaenomene einhergeht, welche die unbelebte Natur bestimmter Virusarten wahrscheinlich machen. Die Elemente des Herpesvirus, des Virus des Rous-Sarkoms und der Hühnerleukosen, des pathogenen Agens der

Mosaikkrankheit des Tabaks und der Bakteriophagen gehören ganz verschiedenen Grössenklassen an und in jeder dieser Klassen kennt man anderseits Virusarten, welche keine wesentlichen Abweichungen von parasitischen Mikroorganismen zeigen. Ordnet man die Virusarten nach fallenden Teilchengrössen, so erhält man also derzeit nicht das Bild des allmählichen Ueberganges lebender Gebilde in Zustandsformen, welche unserer Vorstellung von Organismen nicht mehr entsprechen. Welche Folgerungen aus diesem Sachverhalt abgeleitet werden müssen oder dürfen, lässt sich vorläufig nicht präzis beantworten. Sicher ist aber, dass die Elemente mehrerer Virusarten ein Volumen besitzen, in welchem nur ein einziges oder einige wenige Eiweissmoleküle Raum finden würden. Zweitens mehren sich die Beobachtungen, welche für die reale Existenz von unbelebten endogenen (im Wirtsorganismus entstehenden) Infektionsstoffen (übertragbaren pathogenen Agentien) sprechen. Zu den schon früher bekannten Argumenten sind in letzter Zeit hinzugekommen : (1) Der Nachweis der unbegrenzten Haltbarkeit von Bacteriophagen im feuchten Medium ohne Anwendung besonderer konservierender Massnahmen ; (2) die Darstellung phytopathogener Virusarten in Form krystallisierter Proteine ; (3) die Durchleitung eines phytopathogenen Virus durch die Samen der Wirtspflanze im Verein mit der Tatasche, dass die aus den Samen entstandenen jungen Pflänzchen zunächst virusfrei sind, nach Ablauf von 5-6 Wochen jedoch grosse Virusmengen im Wurzelgewebe enthalten. Vermutlich bestehen hier engere Beziehungen zur Durchleitung von Bakteriophagen durch Bakteriensporen ; (4) die durch Jacchia erneuerte Behauptung, dass die aspezifische Provozierbarkeit des Herpes febrilis auf dem Vorhandensein von Herpesvirus im Liquor beruht, konnte nicht bestätigt werden. Die Sicherheit, mit welcher der Herpes labialis durch Höhenluft hervorgerufen wird, und die Herpesfreiheit der ersten Lebensjahre des Menschen (vgl. Punkt 3) weisen auf den endogenen Ursprung dieses Agens hin.

An dem Grundsatz, dass die Bezeichnung " Virus " ein auf methodologischer Basis entstandener Sammelname für biologsich heterogene übertragbare Agenzien ist, hat man auch bei der Discussion über die Alternative " Belebt oder unbelebt " festzuhalten. Verallgemeinerungen, welche über die Beweiskraft der jeweils bekannten Tatsachen hinausgehen, sind hier ebenso entschieden abzulehnen, wie der Versuch, sämtlichen Virusarten bakteriellen Charakter zuzusprechen.

Dieser Standpunkt wird dadurch nicht erschüttert, dass bestimmte Eigenschaften (z.T. auch miteinander kombiniert) bei einer grösseren Zahl von Virusarten festgestellt werden konnten. Es handelt sich (*a*) um die Unfähigkeit der Vermehrung auf unbelebten Nährmedien, (*b*) um die Bildung von Einschlusskörperchen und (*c*) um die Immunitätsverhältnisse, vor allem um die Produktion der sog. virusneutralisierenden Antikörper.

ad (*a*) Die Unfähigkeit der Vermehrung auf unbelebten Nährmedien kann sehr verschiedene Ursachen haben; dass sie in allen Fällen auf der "Cytotropie" d.h. auf einer Art von obligatem Zellparasitismus beruht, ist nicht nur nicht nachgewiesen, sondern steht auch mit den Erfahrungen der Virusüchtung in Gegenwart von lebenden Geweben (Universalmethoden !) in Widerspruch. Als negative Aussagen haben ferner die Misserfolge der Kultur auf unbelebten Medien nur bedingten bzw. provisorischen Wert. Das gleiche Verhalten wurde schliesslich auch bei Erregern konstatiert, welche man nicht zu den Virusarten rechnet (Rickettsien) und die auch keine obligaten Zellschmarotzer sind (Leprabazillen); umgekehrt ist die Kultur bei den Keimen der Pleuropneumonie und Agalaktie, die wenigstens früher zu den Virusarten gestellt wurden, möglich und Eagles hat seine Angaben für die Vaccine aufrechterhalten.

ad (*b*) Zelleinschlüsse bildet nur ein Teil der Virusarten. Auch sind die Zelleinschlüsse untereinander nach Grösse, Lokalisation, Struktur und Färbbarkeit so verschieden, dass eine einheitliche Auffassung derzeit nicht zu rechtfertigen ist. Infektionsexperimente mit isolierten Einschlüssen sind bisher nur bei einer kleinen Zahl von Virusarten ausgeführt worden und erlauben selbst auf diesem eng begrenzten Arbeitsgebiet nicht die generelle Deutung der "intracellulären Viruskolonien."

ad (*c*) Die Reaktionen zwischen Virus und neutralisierendem Antiserum folgen nicht dem Typus der cytotoxischen Immunphaenomene (Bacteriolyse), sondern erinnern durch die Unabhängigkeit vom Komplement und die Möglichkeit der Aktivierung unwirksamer ("neutraler") Gemische an die Beziehungen zwischen Toxin und Antitoxin. Die Einschaltung der empfindlichen Zellen als Bindeglied in die Reaktion zwischen Virus und seinem Antiserum wurde in ähnlicher Form für das Diphtheriegift und sein Antitoxin beschrieben und findet ein weiteres Analogon im zellständigen Sitz der anaphylaktischen Antikörper und der idiosynkrasischen Reagine im Prausnitz-Küster'schen Versuch. Klargestellt ist der Mechanismus der Virusneutralisation keineswegs und es kann daher auch nicht gesagt werden, dass die serologische Inaktivierung der Virusarten ein Phänomen darstellt, welches auf diese Gruppe von Infektionsstoffen beschränkt ist. Die Produktion von virusneutralisierenden Antikörpern konnte übrigens nicht bei allen Virusarten festgestellt werden und in Beziehung auf die erworbene Immunität zeigen die Viruskrankheiten grosse Unterschiede.

Prof. A. R. Dochez, Dr. Katharine C. Mills and **Mr. Yale Kneeland, Jr.** (New York): ***Studies of Common Cold and Influenza.***

Studies conducted over a period of ten years have revealed that there is a filterable virus present in the upper respiratory tracts of human beings suffering from common colds and influenza.

This virus has a specific relationship to the diseases in question. The presence and activity of these viruses are demonstrated by the experimental production of similar diseases in chimpanzees and human volunteers following inoculations of bacteria-free material which contains the virus. Both viruses can be cultivated for long periods of time, up to one year, in medium containing chick embryo tissue. Such cultures, when inoculated into human volunteers, reproduce regularly symptoms of upper respiratory infection lasting from three to ten days or more. Inoculation of the culture virus of influenza in general does not produce the typical clinical picture of influenza, but one more nearly resembling that of the common cold.

Dr. **H. S. Frenkel** (Rotterdam): *On the Cultivation of the Virus of Foot-and-Mouth Disease.*

The virus of foot-and-mouth disease can be grown easily in the skin of the embryos of the cow and of the sheep. The method of cultivation is based on that of Carrel for vaccine virus and that of Maitland and Cowen Maitland for the viruses of vaccinia and foot-and-mouth disease.

The uterus of a pregnant cow or sheep, which has just been slaughtered, is removed with strict aseptic precautions after cauterization of the skin. The embryo is removed and its skin is immediately minced, first with scissors and then in a mincing machine. Filtered virus and Tyrode's or Ringer's solution is added to the minced tissue. After 2, 3 or 4 days' incubation a 37° C. the culture is tested as usual in the pads of guinea-pigs The titre of the culture rarely exceeds 1 : 1000.

The cultivation of the virus succeeds also if instead of mincing the skin one inoculates it *in situ* by scarification before the embryo is removed from the uterus. Yet another method is to inject the virus into the umbilical vein of the embryo, preferably with a little trypan blue so that the inoculum may be traced to the skin.

Embryo skin after a short treatment with cooled alcohol (55 to 70 per cent.) or after 7 days in cold storage can still be used for the cultivation of the virus or of the tissues (fibrous, epithelial) Bacteria resist the alcohol treatment.

Cattle and pigs have been successfully immunized with the cultures and their serum has shown good protecting power.

Dr. **P. R. White** (Princeton, U.S.A.) [Communicated by Dr R. E. Shope (Princeton, U.S.A.)]: *The Cultivation of Plant Disease Viruses in Actively Growing, Excised Plant Organs* in vitro.

A method of growing isolated plant organs indefinitely *in vitro* in a known nutrient medium and under controlled conditions has been developed and applied to the study of the viruses of certain diseases affecting the tomato. A stock collection of seven such

viruses, which multiply actively under strictly aseptic conditions, is now available. Such cultures are macroscopically symptomless. The rate of multiplication of the virus in the cultures has been shown to be largely independent of the rate of multiplication of the host tissue, although the virus is strictly cytotropic in its active phase. Since the method permits the study of the viruses under simplified controlled conditions in a chlorophyll-free mass of cells which are only slightly differentiated and whose nutrition, including carbohydrate and protein sources, is known and modifiable at will, it offers many potentialities for the study of the physiology of infection by these disease agents.

Prof. K. Herzberg (Düsseldorf): *Die färberische Darstellung von filtrierbarem Virus unter besonderer Berücksichtigung des intracellulären Vermehrungsvorganges.*

Es ist oft versucht worden, in einfacher Weise die Elementarkörperchen so darzustellen, dass ihr Verhalten zur Zelle klar erkannt werden kann und dass die Zelle selbst durch den Färbevorgang möglichst wenig beeinträchtigt wird. Der Farbstoff Viktoriablau 4 R, verstärkt durch einen Zitronensäurezusatz, erfüllt diese Ansprüche, indem er in einzeitiger Färbung, ohne Fixierung oder Beizung, die Elementarkörperchen dunkelviolett bis blauschwarz färbt, dabei Plasma und Kern der Wirtszelle so differenziert und häufig metachromatisch darstellt, dass meist ohne weitere Aufhellungsmethoden die Beziehung des Virus zur Zelle untersucht werden kann. So gelang es mir, einerseits die Elementarkörperchen von 10 Viruskrankheiten mit Viktoriablau zu färben, andererseits den Vermehrungsvorgang in der Zelle beim Virus der Kanarienvogelkrankheit und der Variolavakzine vom ersten Eindringen des Virus in die Zelle bis zu ihrem Zerplatzen zu verfolgen. Dabei ergab sich für das Kanarienvogelvirus, dass der Vermehrungsvorgang in der embryonalen Zelle der Allantois mit der gleichen charakteristischen Vakuolenbildung verläuft wie in den Histiozyten des erwachsenen Vogels. Auch beim Variolavakzine-Virus habe ich die Vermehrung in der Embryonalzelle verfolgt, dort aber einen anderen Vermehrungsvorgang gefunden. Es fehlt hier die Vakuolenbildung. Das Virus vermehrt sich im Plasma unmittelbar, Elementarkörperchen neben Elementarkörperchen. Die Vermehrung geschieht auch hier durch Zweiteilung, und nirgends bieten die Präparate einen Anhalt dafür, das die Vermehrung Entwicklungsstadien durchläuft. An einzelnen Stellen kommt es zu einer Häufung von Elementarkörperchen, die noch von einer tief dunkelblau färbbaren Zellabscheidung umhüllt werden. Für diese schwarzblau gefärbten Elementarkörperhäufchen wurde von Gross im Schnittverfahren nachgewiesen, dass es sich um die Guarnierikörperchen der Embryonalzelle handelt. Auch an der Vakzine-infizierten Kaninchenhornhaut liess sich dartun, dass die Guarnierikörperchen aus einer Zellabscheidung und Elementarkörperchen

bestehen. In letzter Zeit wurde das Ektromeliavirus und das Zostervirus (Zona) in gleicher Weise untersucht.

Prof. S. P. Bedson (London) : *Observations on the Morphology of Viruses with Special Reference to the Virus of Psittacosis and the Bearing of these Observations on the Nature of Viruses.*

The early work on the morphology of viruses was unconvincing because means had not been found of proving that the forms seen were the virus. The use of the centrifuge for this purpose, already indicated by the work of Ledingham with fowl-pox and vaccinia, made possible the identification of the Levinthal-Coles-Lillie bodies with the virus of psittacosis. Levinthal noted that psittacosis virus exhibited pleomorphism. Observations made on this virus, both in stained preparations and in the living state in tissue cultures, have shown that multiplication is associated with morphological changes which follow a definite sequence. The virus grows in the cytoplasm of susceptible cells in circumscribed colonies enveloped in varying amounts of basophilic homogeneous inclusion material. From these studies it is concluded that psittacosis virus is a living micro-organism with an intracellular habit of life, in fact a highly specialized parasite. Morphological evidence suggests that the inclusions seen in trachoma which many consider to be formed by the virus of this disease, are cytoplasmic colonies of a virus which closely resembles psittacosis morphologically. Thygeson has shown that the virus of " inclusion blenorrhœa " and the so-called " swimming bath conjunctivitis " is filterable and that, when multiplying, it exhibits the same sequence of morphological changes as does the virus of psittacosis. It is argued by some authorities that these findings have no bearing on the nature of viruses since the agent of psittacosis and those like it are rickettsia and not viruses. This implies a knowledge of the nature of rickettsia which one does not possess. In any case the methods used in the study of psittacosis virus should be applicable to some of the larger viruses whose nature still remains obscure.

Dr. P. Thygeson (Iowa City, U.S.A.) : *Some Characteristics of the Virus of " Inclusion Conjunctivitis."*

The virus ætiology of " inclusion conjunctivitis," a benign papillary or follicular conjunctivitis characterized by basophilic and heterogeneous inclusions in the cytoplasm of epithelial cells, is indicated by the infectivity of bacteria-free filtrates and by the failure of the agent to develop on artificial media. The infectivity of filtrates depends upon the presence of minute elementary bodies, which form the mature inclusions, a finding which indicates the identity of the virus with the elementary body.

The virus is pleomorphic ; the large form (initial body of Lindner) is similar to the initial body of trachoma and to the large form of psittacosis virus. Both forms stain well with Giemsa's and similar

dyes. The virus undergoes a developmental cycle which is comparable to that of psittacosis virus and is complete in about 48 hours. The presence of well-defined division forms suggests that the bodies are micro-organisms.

Ectodermal epithelium alone appears to be susceptible; natural infection only occurs in the conjunctiva, cervix, and male urethra. The monkey is the only susceptible animal. Attempts at cultivation in the developing egg and in tissue culture have failed.

Neither natural nor experimental infection confers immunity, it has been possible to reinfect the same baboon as many as five times. Neutralizing antibodies or agglutinins are not demonstrable in the blood serum of human cases and there is no skin sensitivity to elementary body suspensions.

Lindner's hypothesis that the virus of " inclusion conjunctivitis " is a mutant of trachoma virus has not been confirmed.

Dr. S. Nicolau (Paris): *Essais de transformation du virus rabique fixe en virus des rues.*

La disparition progressive des corps de Negri pendant la transformation du virus rabique des rues en virus fixe serait due à l'adaptation des germes aux neurones névraxiques : ces derniers n'arriveraient plus à engendrer des inclusions—formations de défense—contre un virus à agressivité exacerbée. Le virus une fois fixé, perd en partie l'habitude de cheminer et de cultiver dans les nerfs périphériques, et, à l'encontre du virus des rues, produit une septinévrite inconstante.

En passant le virus fixe de nerf à nerf, en série, chez le lapin, on le réadapte au système nerveux périphérique, la septinévrite redevient constante et on assiste à la récupération de la " negrigénèse." Le virus ainsi "*ramené en arrière,*" a une incubation plus irregulière chez les lapins inoculés dans le cerveau et produit des corps de Negri dans l'encéphale du chien, comme un virus des rues. Il n'a pourtant pas toutes les propriétés de ce dernier, car il conserve encore les caractères du virus fixe quant aux suites des inoculations cutanées et sous-cutanées.

La transformation du virus des rues en virus fixe et le retour en arrière de ce dernier, seraient ainsi seulement des adaptations plus ou moins réversibles et non pas des mutations fixes, stables, définitives. Dans un virus rabique il y aurait deux fonctions : une, l'affinité pour le névraxe, l'autre, l'affinité pour le système nerveux périphérique. Du jeu de la prépondérance de l'une de ces deux fonctions sur l'autre, jeu dirigé par l'adaptation qu'on accomplit expérimentalement, le virus peut perdre ou acquérir des propriétés qui le font désigner sous le nom de virus fixe ou de virus des rues.

Prof. A. Gratia and **Dr. P. Manil** (Liége) : *La sérologie des virus des plantes.*

(1) L'agent de la mosaïque du tabac et le " virus X " de la pomme de terre sont floculés par les sérums correspondants de

façon rigoureusement spécifique. Il y a donc pluralité des mosaïques. La méthode sérologique permet de les identifier, de les classifier, de dissocier leurs " complexes."

(2) Ces agents sont des antigènes propres, distincts du tissu végétal qui les héberge. Exemple : un sérum préparé contre le jus de tabac atteint de virus X de la pomme de terre floculera le jus de toute plante atteinte de ce virus X quelle que soit l'espèce de la plante ; mais ne floculera ni le jus de tabac normal, ni de tabac atteint de la mosaïque du tabac.

(3) Ces agents sont *autonomes* et conservent leur caractère d'origine quelle que soit l'espèce de la plante à laquelle ils se sont adaptés. Après de nombreux passages sur tabac, le virus X de la pomme de terre reste toujours floculé par le sérum correspondant et ne devient pas floculable par le sérum anti-mosaïque de tabac.

(4) Ces agents ne sont pas transmissibles par les graines. Les sérums correspondants floculent le jus du péricarpe des fruits malades ; mais pas celui des graines qu'ils contiennent, ni celui des plantes issues de ces graines. Il en est ainsi même chez les variétés de pomme de terre " porteuses " de virus X sans symptômes. Le sérum anti-virus X flocule le jus de ces plantes lorsqu'elles sont issues de tubercules ; mais pas lorsqu'elles sont issues de graines.

Conclusion : Les agents des mosaïques sont—même chez les plantes " carriers "—des éléments étrangers non héréditaires ; ils ne peuvent être ni une diastase sécrétée par la plante elle-même, ni un " gène." D'ailleurs, transmis par les insectes, ils ne peuvent être que des virus parasites des cellules végétales.

En raison de l'identité des deux problèmes, la même conclusion doit s'appliquer à la nature des bactériophages.

Dr. E. Wollman (Paris) : *Sur la nature des bactériophages.*

Toute interprétation des phénomènes de la bactériophagie doit tenir compte des données suivantes : (1) la bactériophagie ne se manifeste qu'aux dépens de germes vivants, en voie de multiplication ; (2) elle est liée à la présence d'éléments corpusculaires (bactériophages) ; (3) la reproduction des bactériophages est fonction de la multiplication bactérienne ; (4) les bactériophages n'ont aucune action lytique propre ; (5) ils peuvent apparaître dans des cultures pures ; (6) certains germes produisent des bactériophages actifs pour d'autres germes du même groupe.

Ni les théories diastasiques, ni celle du virus n'expliquent ces faits. Les données récentes sur la grandeur des corpuscules bactériophages et sur leur comportement aux hautes pressions permettent d'éliminer les théories diastasiques. D'un autre côté, la théorie du virus, difficile à concilier avec les faits énumérés sous les alinéas (1) et (3) est incompatible avec ceux des alinéas (5) et (6) et avec les données récentes sur les rapports quantitatifs entre bactéries et bactériophages.

Or, la conception des bactériophages comme supports matériels de certains caractères cellulaires expliqueraient bien ces faits. Ce seraient des *facteurs* lysogènes, au sens des généticiens. Les valeurs trouvées pour la grosseur des corpuscules bactériophages ayant été interprétées en faveur de la théorie du virus, il est intéressant de rappeler que des valeurs de même ordre ont été fournies pour la grandeur des gènes authentiques.

Il est possible que d'autres processus (tumeurs filtrables, certaines mosaïques des plantes) reconnaissent un mécanisme analogue. A côté de processus à virus authentiques (inframicrobes) il y aurait des processus infectieux relevant d'agents d'origine cellulaire.

Prof. R. Bieling (Frankfurt a. M.).

Es muss damit gerechnet werden, dass die reine Virussubstanz an verschiedene, verschieden grosse Trägersubstanzen gebunden ist, so wie z.B. das Tuberkulin natürlicherweise an Globuline gebunden vorkommt, von denen es sich aber trennen lässt.

Der Verteilungsmodus verschiedener Vira im Körper des Infizierten ist verschieden. Während z.B. das Virus der Geflügelpest sowohl bei der weissen Maus (Ørskov) wie beim Kanarienvogel sich lange Zeit im Blut hält, verschwindet das Virus des Louping-ill selbst nach i.v. Infektion sehr rasch aus dem Blut.

Prof. R. Bruynoghe (Louvain).

Divers auteurs ont signalé que les bactériophages ne se multiplient que pour autant qu'il y ait développement des microbes sensibles.

Nous avons établi ce fait en soumettant à l'action des bactériophages des microbes, en l'occurrence des bacilles typhiques, irradiés au radon. À la suite de cette irradiation les microbes perdent la propriété de se multiplier et de se diviser, tout en conservant encore durant 8 à 10 jours leur vitalité et notamment leur mobilité.

Ces microbes vivants mais impropres à la culture ne permettent pas le moindre développement du bactériophage, ce qui confirme d'une façon évidente le fait signalé d'abord par J. Bordet que la multiplication du bactériophage ne se fait que pour autant qu'il y ait culture, c'est à dire développement des microbes sensibles.

Prof. J. Bordet (Brussels).

Comme au Prof. Doerr, il m'est arrivé de conserver des principes bactériophages pendant 10 à 12 ans, et de constater non seulement que le principe avait conservé toute son activité, mais qu'il se reproduisait en présence de la bactérie sensible aussi rapidement qu'un principe tout récemment obtenu. D'autre part on obtient parfois en soumettant à la technique de l'isolement des bactéries lysogènes, des colonies isolées qui ne possèdent pas ce pouvoir lysogène. Mais comme je l'ai signalé en 1923, ces colonies inactives

fournissent des subcultures qui, repiquées en série pendant un temps assez prolongé, redeviennent actives. Il est vrai que les partisans de la théorie du virus peuvent objecter que les colonies isolées contenaient du virus sous une forme très atténuée et que ce virus était capable de reprendre sa virulence après avoir été cultivé en présence de la bactérie pendant un temps suffisant.

Dr. S. Marbais (Livry-Gargan, France) : *Inexistence du bactériophage.*

La mesure de la pression carotidienne dans l'anaphylaxie et dans l'immunité avec le sphygmomanomètre m'a donné l'occasion de découvrir une réaction spécifique que j'utilise dans l'étude de la question du bactériophage. J'ai pu inférer que le bactériophage n'existe pas en tant qu'organisme vivant et autonome.

(1) En injectant des lapins avec une culture de bacilles de Shiga et en mesurant la pression artérielle spécifique de choc nous constatons que la pression baisse aussi bien après l'inoculation de bacilles de Shiga que du bactériophage de ce bacille. Elle ne baisse pas après l'inoculation du bouillon nature, ni du bacille pyocyanique ni du bactériophage pyocyanique, pris comme témoins.

(2) À la phase d'immunité la pression augmente après l'inoculation d'épreuve du microbe non lysé et aussi de son bactériophage.

(3) En inoculant des lapins avec du bactériophage on les sensibilise ou on les immunise pour cet antigène, mais en même temps, pour les microbes non lysés d'où ils proviennent. La pression baisse ou augmente parallèlement apres l'inoculation seconde de bactériophage ou de son bacille non lysé.

(4) Pour voir si dans le bouillon-bactériophage il y a effectivement deux antigènes différents, j'ai sensibilisé des lapins avec le bactériophage Shiga. La veille de l'épreuve j'ai vacciné quelques lapins du lot avec du Shiga non lysé. Or l'inoculation d'épreuve du Shiga non lysé faisait monter la pression artérielle du lapin vacciné, ce qui est normal. Mais l'inoculation du bactériophage Shiga la faisait monter également, ce qui ne devait pas avoir lieu si dans le bouillon-bactériophage il y avait un antigène différent de la structure protéique du bacille de Shiga, antigène contre lequel je n'avais pas vacciné les lapins.

Ces quatre types d'expériences nous montrent que la constitution antigénique du bactériophage est la même que celle de son microbe, et que par conséquent le " bactériophage " n'existe pas.

Prof. R. Doerr (Basel).

Dass die serologischen Reaktionen, welche Gratia bei den phytopathogenen Virusarten beobachtet hat, den Typus von Präzipitationen und nicht von Agglutinationen zeigten, steht mit der Auffassung von der mikrobiellen Natur dieser Agentien in Widerspruch. Die Annahme von Bordet, dass jeder Bakterienstamm, bei welchem die Lysinproduktion erstmalig in Erscheinung tritt, schon vorher

mit Bakteriophagen "*latent infiziert*" war, ist eine Hypothese, die man in allen ähnlichen Fällen, allerdings, nur willkürlich, anwenden kann, z.B. beim provozierbaren Herpes oder bei den Beobachtungen von K. Smith über die Durchleitung von Virus durch die Samen von Tabakpflanzen. Man darf jedenfalls in der Verwertung solcher Hypothesen nicht einseitig und inkonsequent sein und sie nur dann heranziehen, wenn sie eine Auffassung stützen sollen; wenn Gratia die Unmöglichkeit der Virusübertragung durch die Samen als Argument für die belebte Natur der Virusarten benützt, kann man nicht die nachgeweisene Möglichkeit solcher Phänomene beiseite schieben.

Dr. M. Schlesinger (London).

Some information concerning the number of phage-particles arising from the union of a single bacterium with one phage-element is provided by the following experiment: (*a*) 1 c.c. of broth containing 10^9 bacteria and 1 c.c. of broth containing 10^9 phage-particles are mixed. Union occurs immediately. The mixture is then diluted 10^7-fold.

(*b*) 10^7-fold dilutions are made of the organism and of the phage separately and these dilutions are mixed. No union can occur under these conditions.

Both systems are incubated. After 2½ hours no change is found in the phage-content of (*b*). On the other hand in (*a*) 2-, 4- and 10-fold multiplication occurs after 30, 60 and 90 minutes.

Similarly if (*a*) is divided into equal samples containing on an average one single phage-bacterium complex and these are incubated separately and tested in groups, we find after 30 minutes no multiplication at all in 95 per cent. of the samples, whereas in the remaining 5 per cent. the number of plaques produced is 10–40, *i.e.* on an average 20 (instead of 1). This sudden increase appears in about 20 per cent. of the samples after 60 minutes, and in 50 per cent. after 90 minutes, etc. These results are in general agreement with the earlier findings of Burnet; but they are obtained under conditions where the possibility of the combination of the newly-formed phage-elements with other bacteria is excluded, and the sudden 20-fold increase can safely be attributed to a single phage-bacterium complex.

DISCUSSION *on* THE GENERAL CHARACTERISTICS OF VIRUSES, INCLUDING BACTERIOPHAGE

Second Day.

Chairman: Dr. J. Henderson Smith (Rothamsted, England).

Dr. J. Henderson Smith (Rothamsted), **Opener.**

It is generally agreed that the viruses which occur in plants are the same in kind as the viruses found in animals. The difference

of environment in the two classes of viruses must influence their behaviour and the effects produced by their presence ; but there are problems, especially those connected with their nature, which are common to both types, and some of these problems are more readily attacked in plants than in animals. With plants it is easy to use large numbers, even hundreds, but this is impossible in animal work through considerations of space and expense ; and the results of experimentation can be placed on a quantitative basis. The " local lesion " technique affords an exceptionally convenient and valuable method for the investigation of many problems (e.g., the action of chemical or physical agents on a virus) with a precision and on a scale hardly practicable in animals. It is possible to obtain without difficulty large quantities of virus-holding liquids ; and methods of purification of the active agents from accompanying extraneous material have been worked out with considerable success.

It was at one time believed that plant viruses were highly specific in their range and were confined, if not to one plant, at least to members of one family. This generalization has had to be abandoned. Several viruses have an astonishing width of range: for instance, the virus of the curly-top disease of sugar-beet can infect 300 species of plants belonging to 33 different families, and the virus of aster yellows can infect 170 species from 38 different families. There are still some viruses, e.g., mosaic of sugar-cane, whose range is apparently restricted, but improvements in technique are extending the range even of these ; and, speaking generally, one may say that specificity of range is the exception and not the rule. It is not practicable in animal pathology to test the susceptibility of 200 or 300 species to a single virus. But progress depends very largely on the discovery of a susceptible animal, and if the plant analogy holds good we may expect that other hosts are discoverable even for those diseases known only in the animals in which they naturally occur, and there is almost as much likelihood of finding these susceptible animals in remote as in kindred species. The symptom-syndrome may have very little relation to the identity of the virus concerned, and the same virus may produce wholly different effects in different hosts. Conversely, there is no argument for identity of cause in similar symptoms. Environmental conditions, such as temperature and food, have a marked effect on the expression and even the incidence of virus disease in plants; they may determine the local or systemic character of an infection, and in the limiting case they may produce the carrier state in which the virus is present in a virulent form without showing signs of its presence. Other instances might be given of the establishment in plants of facts, of which the general validity is still unproved on the animal side. It is sufficiently evident, however, that in our knowledge of viruses, plant pathology has already made contributions whose scope is not confined to plants, that there are questions asked in animal pathology which can be answered more

easily and with greater wealth of illustration in plants, and that closer contact between the animal and the plant side of virus research will be of immediate advantage to both.

Mr. J. E. Barnard (London) : *The Microscopy of Viruses.*

From a microscopical standpoint it is convenient to regard a virus as an organism the size of which is less than the resolving limit imposed by the microscope when one uses visible light. Such a limit is reached with a body or element of structure smaller than 0·2μ in any dimension. The main factor determining resolution in the microscope is the wave-length of light used as an illuminant. On physical grounds, therefore, it should be possible to obtain an image of any virus at present known, whatever size may be assigned to it, since the range of radiations extends far beyond the possible limits of size. On optical grounds, however, it is not yet practicable to utilize radiations in the ultra-violet region beyond the limit of transmission through quartz, and incidentally through air. A microscope fitted for ultra-violet radiations can only produce a photograph; no image of value can be obtained visually. Considerable advances have been made both on the mechanical and optical sides, the former ensuring higher accuracy of adjustment, without which the full value of optical improvements is not secured. Many practical difficulties arise in the application of this method to virus work, not the least of which is the preparation of infective material so that bodies which are suspected to be virus can be mounted in such a way and in such a medium that photography is possible. A short account was given of the apparatus devised for this work and now used at the National Institute for Medical Research, and some of the results obtained were shown.

Dr. W. J. Elford (London) : *The Particle Sizes of Viruses and Bacteriophages in Relation to Bacteria and Proteins.*

Considerable evidence has accumulated on the particle size of viruses and bacteriophages through ultra-violet light photography, centrifugation analysis and ultra-filtration analysis. Excellent agreement is in general shown among the figures furnished by the different methods. The particle sizes of viruses range from 200 mμ down to 10 mμ, and those of bacteriophages from 75 mμ to 10 mμ. The smallest organisms so far cultivated in cell-free media (viz., bovine pleuro-pneumonia and the new group of organisms recently isolated from sewage) have developing particles as small as 150 mμ, while some proteins (e.g., hæmocyanins) may have particles as large as 24 mμ in diameter. Thus the scale of viruses and bacteriophages is overlapped at its upper end by certain cultivable organisms and at its lower end overlaps the larger proteins. The failure to cultivate at least some of the larger viruses cannot be explained by

particle size alone, yet the fact that some viruses are smaller even than certain proteins prompts the query " Can such a small, compact structure function as a living entity ? " Most known viruses come within the range of 200 mμ to 75 mμ, and here ultra-violet light photography supports the view that viruses are indeed small micro-organisms. On the other hand, the view that the smaller viruses and the bacteriophages are produced by the cell through some derangement of its normal intracellular processes, as a result of a primary infection, cannot be gainsaid on the evidence of particle size.

Mr. I. A. Galloway (London) : *Purification of Viruses.*

Until quite recently attempts to purify viruses have been based chiefly on their surface properties, which in general are very similar to those of other substances in a state of colloidal dispersion, e.g., reversible adsorption on to surfaces of opposite electric charge and migration in an electric field. Separation by these methods is therefore extremely difficult, and the final product is invariably of low virus potency. Alternative methods have now been devised as a result of the knowledge acquired on the sizes of the different viruses, viz., washing processes based on differential centrifugation and filtration.

By centrifugation methods preparations have been obtained of two of the largest viruses, those of vaccinia and psittacosis, largely freed from extraneous protein with little loss of potency. This method has also been applied with success to one of the larger bacteriophages, but with the ordinary types of centrifuge and with the usual technique it cannot be applied successfully to viruses or phages smaller than 100 mμ. However, we have now obtained evidence that by spinning in the closed, agar-lined (Schlesinger) cylinder of a Sharples centrifuge one can throw down even the smallest known viruses (e.g., foot-and-mouth disease = 8 – 12 mμ by ultra filtration) and phages (e.g., S13 phage = 8 – 12 mμ).

The 100 to 10,000-fold drops in titre of the supernatant fluid obtained after different periods of centrifugation, 5 – 30 mins., are entirely due to centrifugal force and have been shown to bear no relationship to adsorption phenomena. This centrifuging method can also be used for purification, for by spinning down the virus and subsequently extracting the agar in fresh buffered phosphate solution a preparation is obtained of equal potency to that of the original, and partially freed of protein. It is not advisable, however, in the case of the virus of foot-and-mouth disease at least, to carry the washing process in the centrifuge beyond a certain limit since we have found that highly purified preparations of virus may be adsorbed by the agar.

Purification by filtration, i.e., carrying out the washing on a membrane of suitable porosity, has a wider application and has been

used with success for purifying enzymes, the agent of Rous-Sarcoma No. 1, bacteriophages and recently one of the smallest viruses, that of foot-and-mouth disease.

It is difficult to use collodion membranes for washing virus suspensions of very high protein content, but these suspensions can be dealt with in the Sharples centrifuge. By a combination of the two methods, *i.e.*, washing once in the centrifuge to reduce the protein content and then repeatedly on a collodion membrane, very potent purified concentrates of virus can be rapidly obtained, especially if the final concentration is made on filter candles coated with 9 per cent. acetic acid collodion. In this way concentrates of foot-and-mouth disease virus having a titre of 10^{-8} and giving a negative sulpho-salicylic acid test for protein have been prepared in one day from virulent vesicle lymph (diluted 1 : 2) having a titre of only 10^{-6}.

Dr. W. M. Stanley (Princeton, U.S.A.): *Crystalline Tobacco-mosaic Virus Protein.*

A crystalline protein, which has the properties of tobacco-mosaic virus, has been isolated from extracts of Turkish tobacco plants infected with this virus. The virus activity, chemical composition, and optical rotation of crystalline protein obtained from many different lots of starting material are the same. These properties remain constant on recrystallization of the protein ten times, on fractional crystallization, or on fractionation by adsorption on, and elution from, celite. When solutions are made more alkaline than pH 11, or more acid than pH 1, or are heated to about 80° C., the protein is denatured and the virus activity lost. The protein is not digested by trypsin, but is slowly digested by pepsin, with loss of virus activity. Its isoelectric point is about pH 3·2 and its molecular weight is greater than that of any other known protein. One cc. of a solution containing 10^{-9} g. per cc. of the crystals is usually infectious. The sera of animals injected with extracts from diseased plants or with solutions of the protein give a precipitate when mixed with solutions containing 10^{-6} g. per cc. of the crystals.

Crystalline protein, possessing similar physical, chemical and biological properties, has been isolated from extracts of tomato plants infected with tobacco-mosaic virus. Two additional crystalline proteins have been isolated from extracts of Turkish tobacco plants infected with a masked strain (Holmes) and a yellow (aucuba) strain of tobacco-mosaic virus respectively. These two proteins, although apparently related to each other and to the crystalline protein from plants infected with ordinary tobacco-mosaic virus, possess properties that distinguish them from each other and from the tobacco-mosaic protein.

Dr. M. Schlesinger (London): *Pure Bacteriophage prepared in Weighable Quantities.*

By cultivation with a strain of *B. coli* under certain conditions on a Na-lactate-NH_4Cl medium, the bacteriophage " WLL " (size by centrifuging 90 mμ) reaches a titre of 2×10^{10} and over. The clear, colourless filtrate shows a Tyndall effect which is due to the phage content as proved by parallel nephelometric measurement and phage-count (after centrifuging, filtration, specific absorption, etc.). The particles which scatter the light can be resolved by the cardioid ultramicroscope. They are very uniform in size, show rapid Brownian movement, are spherical (Szegvari-diaphragm), correspond in number to the titre and are specifically agglutinated by antiphage serum. By concentration on collodion membranes, preparations containing 10^{13} active particles and 10 mg. of dry phage substance per cc. can be obtained, and after repeated washing in the centrifuge at 15,000 r.p.m., they are free of bacterial antigen, and remain unaltered in their composition and properties by further washing. The dry weight of a single particle is about 4×10^{-13}mg., and the water-content is less than 50 per cent. Analysis: C 42 per cent.; H 6·4 per cent.; N 13·2 per cent.; P 3·7 per cent. The biuret, xanthoprotein and Millon's tests are positive. Molisch's test is negative. Lipoids are present. A high affinity for basic dyes and the P-content remind one of chromatin.

Schüler, working with the author's preparations, showed that there was no O_2-consumption or CO_2-production even in a sample containing 4×10^{13} particles (equal to 20 mg. dry weight), but he found a high phosphatase content. No other ferment was detected.

The ultramicroscopic findings mentioned above demonstrate directly the fact derived in 1932 from centrifuging experiments, viz., removal of the active units up to 99·999 per cent. by repeated spinning without any shift of the sedimentation rate, that the phage units are morphological elements of a uniform and characteristic size and shape. The view that their chief constituent is of a nucleoproteid nature is supported by the recent finding that the phage substance is stained intensely by Feulgen's reagent, but bacteria and bacterial débris, treated in the same way, remain unstained.

Dr. K. M. Smith (Cambridge): *The Problem of a Plant Virus Infection.*

A plant virus possessing some unusual characteristics has been discovered. It is present in the roots of a large number of different species of plants all of which, with the exception of *Nicotiana tabacum* and *N. glutinosa*, remain normal in appearance. The virus has only been found under glasshouse conditions. It differs from other plant viruses in withstanding 99 per cent. alcohol for 13 days and in its capacity to remain infective in the

and in the absence of living tissue for six to nine weeks or longer. It can be completely dried and kept in a test tube for long periods.

Evidence has been obtained which shows some connection between the virus and the soil but the exact relationship has not yet been determined. The chief discrepancy lies in the fact that while only excessively minute quantities of the virus can be demonstrated in the soil, yet tobacco plants, if grown in the same soil, frequently show 80–100 per cent. infection of the roots with the virus. Plants, previously shown to be virus-free, become infected with the virus whether grown in unsterilized or autoclaved soil. It appears therefore as if the virus is either air- or water-borne and so reaches the soil whence it makes its way to the roots of the plant.

There seems little doubt that the method of dispersal of this virus differs from that of other plant viruses and resembles the method of dissemination of fungi and bacteria.

Dr. G. Hardy Eagles (London): *Acute Rheumatic Fever and Rheumatoid Arthritis as Virus Diseases.*

In 1935 the published report of an investigation by Schlesinger, Signy and Amies pointed very strongly to a virus agent in acute rheumatic fever. It seemed probable that similar lines of investigation might yield evidence of a virus ætiology in such rheumatic conditions as rheumatoid arthritis and acute chorea.

Pericardial and pleural exudates and joint fluids from acute rheumatic fever and joint fluids and synovial membrane from acute and sub-acute rheumatoid arthritis have yielded suspensions of elementary bodies which in stained preparations and by dark-ground examination are indistinguishable from elementary bodies demonstrable in similar suspensions from recognized virus infections. Synovial membranes contain relatively few of these bodies, but numerous other bodies of varying size which stain indifferently. Control materials from cases where a virus ætiology is not suspected contain similar particles of varying size from which suspensions may be prepared.

Agglutination tests in hanging drop preparations have shown that suspensions from cases of rheumatic fever are agglutinated by sera from patients in the acute phase of rheumatic fever (carditis, early nodule formation, etc.). These rheumatic fever sera have also agglutinated suspensions prepared from joint fluid in acute and sub-acute rheumatoid arthritis in a number of cross-agglutination tests. Some evidence of cross-agglutination has also been obtained between early acute rheumatoid sera and suspensions from acute rheumatic fever. Control suspensions remain unagglutinated by all sera, and normal sera do not agglutinate any suspensions tested. Spinal fluids from cases of chorea have in one instance only, yielded anything comparable to elementary bodies.

All suspensions are being tested against sera from cases of acute chorea.

Evidence so far obtained suggests a possible virus ætiology in rheumatoid arthritis and some relationship to acute rheumatic fever.

Dr. F. Gerlach (Mödling bei Wien) : *Beobachtungen bei der in Österreich auftretenden Psittakose.*

Infektionen mit Psittakosevirus sind keineswegs immer Anlass zu schweren oder gar tödlich verlaufenden Erkrankungen an Psittakose bei anfälligen Individuen (Menschen und Tieren). Leichte, in Ausheilung übergehende Erkrankungen beim Vogel und Menschen sind häufige Vorkommnisse.

Ebenso wie es Psittakose-Virusträger unter Papageienvögeln und weissen Mäusen gibt, kommen auch beim Menschen Fälle vor, in denen sich als Folge einer Infektion mit Psittakosevirus Erscheinungen einer Erkrankung *nicht* entwickeln. Es ist gelungen, beim Menschen Träger von Psittakosevirus nach "stummer" Infektion mit dem Virus der Psittakose ausfindig zu machen. In einem solchen Falle war das Virus 75 Tage lang im Sputum nachzuweisen. Die Gefährlichkeit von Menschen für ihre Umgebung, die als Virusträger das Virus ausscheiden, ist erwiesen.

Der aus menschlichen Virusträgern isolierte Ansteckungsstoff der Papageienkrankheit unterscheidet sich weder morphologisch noch biologisch (in Virulenz und Pathogenität) von Psittakosestämmen, die von schweren und selbst tödlich verlaufenden Erkrankungsfällen stammen.

Es gibt demnach Menschen, die, ohne zu erkranken, einer Infektion mit virulentem Psittakosevirus zu widerstehen vermögen. Zu entscheiden bleibt, ob es sich dabei um eine dauernde natürliche Resistenz gegen Psittakose handelt, oder um eine befristete, die zu einer anderen Zeit erlischt, bezw. nach Aenderungen in der individuellen Disposition einer Infektionsbereitschaft Platz macht.

Von grosser Bedeutung wäre es, ein Bild über die Verbreitung solcher gegen Psittakose natürlich resistenter Menschen zu erlangen.

Das Problem der künstlichen Schutzverleihung gegen Psittakoseinfektionen wäre nun namentlich im Interesse des meist gefährdeten Personals von Psittakoseabteilungen in Spitälern und Laboratorien erneut unnachgiebig zu verfolgen, da das Vorkommen einer natürlichen Resistenz gegen die Psittakose die Annahme rechtfertigt, dass sich auch eine erfolgreiche aktive Immunisierungsmethode gegen diese Krankheit ausfindig machen lassen dürfte. Da wir aus der neuesten Veröffentlichung von *Pfaffenberg* wissen, dass nach Spontanerkrankung des Menschen an Psittakose kein lebenslänglicher Schutz gegen Reinfektion zurückbleibt (2 Fälle), wird wohl mit einer Impfmethode das Auslangen gefunden werden müssen, die einen zeitlich begrenzten Impfschutz herbeizuführen vermag.

Mr. F. C. Bawden (London).

By a method different from that used by Dr. Stanley, Mr. Pirie and I have isolated protein crystals from plants of different species infected with tobacco mosaic virus. This protein precipitated with anti-tobacco mosaic serum at a dilution of $1/6 \times 10^6$ and was infective at a dilution of $1/10^9$. From the sheen shown by this protein in solution it is probable that the molecule is rod-shaped and not spherical.

Prof. A. v. Jeney (Debrecen, Hungary).

In 1927 I described a bacterial strain under the name of *B. cloacæ Szeyediensis crystalliformans*. This strain showed signs of the presence of a phage (bacteriolysis, "taches vierges," "Flatterformen") and a peculiar form of disintegration of the colonies not previously described. In addition an outstanding feature was the formation of rhomboid crystals underneath the disintegrating colonies and especially in the lytic zones. The formation of these crystals was independent of the salt content of the medium. I concluded that these crystals originated under the action of the bacteriophage from the disintegrated bacteria, but I failed to separate them for chemical investigation.

Dr. R. N. Salaman (Cambridge).

I should like to ask Dr. W. M. Stanley the following questions :—

(1) Did he try out the following, viz., to adsorb a solution of his crystals with anti-tobacco mosaic serum, throw down the precipitate and then attempt once more to obtain the crystals in the supernatant ?

This is on the supposition that the crystals might be broken-down material to which virus particles adhere in some way and that the former is not necessarily antigenic.

(2) Is the actual quantity of crystals obtainable from plants infected with common mosaic, yellow mosaic and masked mosaic, more or less the same and, if not, what are the relative amounts ?

From the point of view inherent in the above suggestion one would expect to obtain considerably less crystals from the plants with masked mosaic than from either of the other two.

Dr. W. M. Stanley (Princeton, U.S.A.).

A majority of the protein in mosaic-diseased Turkish tobacco plants has been isolated in the form of crystalline tobacco mosaic virus protein. This may have some bearing on the question of the nature of X-bodies.

Until tobacco mosaic virus is grown on cell-free media there may be some doubt as to whether or not it is reproduced by itself or by the plant.

The virus activity sediments on centrifugation at the same rate as the crystallisable protein of high molecular weight.

I understand that the typical virus protein has been recovered from so-called "neutral" antigen-antibody complexes.

Prof. A. Gratia (Liége).

The behaviour of crystallized proteins isolated by Stanley from mosaic-infected plants and the antigenic properties of such plants which we reported here yesterday, are quite parallel. As we failed to obtain precipitins against virus "Y" of potato and against sugarbeet mosaic I suggest that Dr. Stanley or Dr. Bawden extend their chemical researches to these viruses also.

Agar cultures of certain strains of bacteria when acted upon by bacteriophage give rise to the formation of crystals. Crystals have also been described by Dufresnoy inside the cells of mosaic-infected plants. Although they are probably by-products it would be interesting to know what relation they might have to Stanley's crystals.

Crystallization of plant viruses had already been claimed by Barton Wright and McBain but in the form of protein-free crystals. Their results have been criticized by Caldwell.

The important thing is to know if mosaic crystals grow by themselves, the plant being passive, or if they are actively produced by the plant.

Dr. C. E. van Rooyen (Edinburgh).

Preliminary investigations have been commenced at the Bacteriology Department, Edinburgh University, in an attempt to confirm recent work regarding the virus theory of rheumatic fever.

In collaboration with Dr. C. A. Green and Dr. J. Sclater, I have examined microscopically a number of serous exudates from rheumatic and non-rheumatic patients for the presence of elementary (or Paschen) bodies.

No significant differences have as yet been detected between fluids from rheumatic subjects and control materials examined at the same time.

Bacteriologically sterile pericardial fluid and joint effusions obtained at biopsy or necropsy from cases of acute rheumatism have been centrifuged at high speed (15,000 r.p.m.), the deposit suspended in saline and then inoculated into human subjects.

Thus, patients suffering from acute, subacute and chronic rheumatism together with normal individuals, have been inoculated both subcutaneously and intradermally and then placed under close clinical supervision. As yet no local, or general reactions have been observed after such injections. Monkeys and other laboratory animals have likewise been inoculated with negative results.

From the initial results obtained there appears to be no alternative, but to conclude that the exudates used for human inoculation showed no evidence of pathogenicity for man. These experiments are being continued.

Dr. M. Schlesinger (London): *A New Centrifuging Technique with greatly increased Effect.*

Principle.—After travelling a distance many hundred times shorter than under the usual conditions of centrifuging the suspended particles are held mechanically, and not by adsorption, in the pores of a thin gel-layer lining the centrifuge tube.

Experimental procedure.—5 to 10 c.c. of liquid agar (1 to 2 per cent.) are introduced into the warmed, closed cylinder of the Sharples Super Centrifuge. This agar sets as a uniform cylindrical film of 0·2 to 0·4 mm. thickness while the centrifuge is running. The centrifuge is stopped and 2 to 20 c.c. of virus suspension (or other liquid to be centrifuged) are pipetted into the agar-lined cylinder. This amount of fluid when spun forms a layer having a thickness of only 0·08 to 0·8 mm. After centrifuging, the supernatant fluid can be easily pipetted off from the agar layer which contains the deposit.

Example of results.—With a centrifugal force of 20,000 times that of gravity, 99 per cent. of the virus is removed from 5 c.c. of diluted foot-and-mouth disease vesicle lymph in five minutes. If the agar layer is extracted in the supernatant fluid and all traces of the agar are removed by filtration the resulting liquid has the same virus titre as the original diluted lymph. (Experiments with I. A. Galloway.)

Applications of the method.—(1) Estimation of particle size from (*a*) sedimentation rate and (*b*) sedimentation equilibrium.

(2) Estimation of the specific gravity by spinning in media of known viscosities and densities.

(3) Concentration and purification (*see* remarks by I. A. Galloway).

Dr. J. Ørskov (Copenhagen) also took part in the Discussion.

DISCUSSION *on* MODES OF TRANSMISSION AND PATHS OF INFECTION IN VIRUS DISEASES

Chairman: Dr. T. M. Rivers (New York).

Dr. E. Weston Hurst (London), **Opener.**

Although most neurotropic viruses reach the brain by way of the peripheral nerves, and probably by the axons, the virus of equine encephalomyelitis at least does not utilize this route. While circulating in the blood, it passes out on to the nasal mucosa, and

the frontal region of the brain is the first part of the central nervous system to become infective. It was argued that this virus must travel by the perineural lymphatics of the olfactory nerve, and the bearing of this observation on certain other recently published work was considered.

Dr. Max Theller (New York) : *The Affinities of Yellow Fever Virus for Various Tissues of Several Experimental Animals.*

All the strains of yellow fever virus so far isolated and studied (22 in number) possess neurotropic affinities and as a rule produce encephalomyelitis in mice, when injected intracerebrally. The infection with this virus in mice is confined to the nervous tissues. In addition to its inherent neurotropic property, the virus of yellow fever also produces a generalized systemic infection in rhesus monkeys, with a selective localization in the liver. By brain-to-brain passage in mice, the virus gradually loses it viscerotropic property; that is, a highly virulent virus becomes converted into a relatively avirulent one. A comparative study of the course of infection in rhesus monkeys inoculated with unmodified virus as well as with the mouse virus shows that the viscerotropic virus multiplies chiefly in the liver, whereas the neurotropic virus produces only a mild systemic infection in which the virus multiplies in the lymphoid and hæmopoietic tissues without selective localization in the liver. Occasionally monkeys inoculated extraneurally with the modified virus develop, after a mild systemic infection, an encephalomyelitis which in spite of the presence of demonstrable protective antibodies in the blood proves fatal. In such animals the virus at death is confined almost entirely to the nervous tissues.

Dr. W. S. Gordon (Edinburgh) : *Louping-ill, a Virus Encephalomyelitis of Sheep.*

Until recent years, the pathogenic filterable viruses have generally been classified according to the post-mortem pathology of the diseases which they cause. By this method the virus of louping-ill would fall into the strictly neurotropic group, since, in the final stages of this disease, the essential pathology is that of an encephalomyelitis. Louping-ill, however, is not exclusively a disease of the nervous system, especially from the outset. The natural mode of transmission is by the bite of infected ticks, the vector being *Ixodes ricinus* (L.). In the early stages of infection the virus probably multiplies in the blood and spleen. Invasion of the central nervous system by the virus occurs at a late stage in the infection, and is responsible for the characteristic symptoms of the disease. Accordingly, its classification as a pantropic virus, type 2 (Hurst) is to be preferred to that of a strict neurotrope.

Dr. W. Maldwyn Davies (Bangor): *The Ecology of the Aphis Vector* Myzus persicæ *Sulz in Relation to the Dissemination of Virus Diseases of Potato.*

The spread of virus diseases in plants is associated with the ecology of the vectors and particularly with factors affecting their migration and subsequent reproduction within the crop in a given season.

The source of the vector before the appearance of an annual crop affects the dissemination of disease. In North Wales the insect vector *Myzus persicæ* hibernates in quantity in the apterous stage on winter cruciferae. The intensity of the initial infestation is influenced by the proximity of large areas of winter host plants.

Meteorological conditions at the period of migration of winged aphides from their winter hosts to the potato crop influence the degree of the infestation. High humidities inhibit flight, and the population is greater in dry districts. Wind velocities above 4 m.p.h. prevent migration of alatæ, which attains a maximum when light breezes come from dry quarters.

Winged *Myzus persicæ* readily find isolated potato plants.

The spread of virus diseases by the winged migrants is negligible, for, in the field, infection among them is rare.

The constant movement of the apterous individuals is a major factor in the spread of disease within the crop.

Suitable districts, where conditions do not favour severe infestations of the vectors, should be selected for the production of healthy stocks. There is a significant difference in the aphis population in districts where seed potatoes have been produced for eight successive years without appreciable increase in virus infection as compared with districts where seed potato production has been discontinued owing to the rapid spread of virus diseases in the stocks.

Standard methods for ascertaining the population of vectors in different districts must be evolved for comparative field surveys.

The ecology of vectors of virus diseases should receive greater attention.

Dr. H. H. Storey (Amani, East Africa): *The Transmission of Plant Viruses by Insects.*

Insect vectors are of paramount importance in plant pathology. The mechanism of the transmission process has been studied on *Cicadulina* spp. and the virus of maize streak disease that they transmit.

Susceptible and immune individuals, able and unable respectively to transmit, occur naturally in all three species of *Cicadulina* studied. In one species susceptibility is controlled by a dominant factor, linked with sex. In the other species also susceptibility is known to be inherited.

After puncture of the insect's abdomen, ingested virus appears in the body fluid of a susceptible individual but not in that of an immune one. An immune insect may become infective if virus is inoculated into the body-fluid, or if its intestine is punctured before or after a feed on the virus. Immunity thus depends on a property of the intestinal wall that prevents passage of the virus into the body fluid.

Virus must reach the body-fluid to render an insect infective and, in the general view, it is then ejected with the saliva, but all attempts to demonstrate virus in the saliva have failed.

Studies of the process of inoculation have shown that infection of a plant is determined during the maintenance of a puncture ; that for success the puncture must penetrate to the phloem and must be maintained for a certain time. The insect's saliva is ejected during penetration, but not during suction, and it sets to a firm gel immediately after ejection. It is difficult to reconcile the view that this material is the vehicle of the virus inoculation with the facts already mentioned.

Prof. Takashi Matsumoto (Taihoku) : *Some Serological Studies on Plant Viruses and Bacteriophage.*

The serological method has been used in studying the distribution and concentration of tobacco mosaic virus in different parts of infected plants as well as in identifying or differentiating various plant viruses and some strains of bacteriophage obtained from *Bacterium solanacearum.* For the first problem the author's micro-serological methods were used. The virus passes out from the young inoculated leaf usually within two days when kept at 28° C., this time varying according to the temperature and other conditions, and travels to the top and the root almost simultaneously, though usually earlier to the top. As has already been noticed, there is more virus in the paler areas of the mosaic leaf than in the dark green areas or midribs, and it is as concentrated in the xylem portion as in the cortex, but it becomes weaker in the former with age. In the flower buds, upper stems and sepals there is less virus than in the bud leaves, basal stems and petals respectively. The stamens and pistils contain very little virus.

The various plant viruses were differentiated by the precipitin test, but for the virus complex the precipitin absorption method was employed. By these means the following viruses could be definitely differentiated : tobacco mosaic, petunia mosaic A, potato mosaic, sugar-cane mosaic, tobacco etch-type, one type of tobacco vein-banding, etch and vein-banding complex, tomato streak (the virus complex), etc. As regards the bacteriophage, the lytic agents were found capable of stimulating the production of specific neutralizing antibody, but unable to produce precipitin.

Dr. J. Ørskov (Copenhagen): *Paths of Infection with Certain Viruses. Effect of Adsorption on Alumina.*

Two strains of fowl-plague virus, studied with S. Schmidt, possessed nearly the same virulence for fowls, but not for mice. One strain killed mice regularly in dilutions of 1–50,000 by the subcutaneous route, while the other strain did not kill them even in dilutions of 1–10. A systematic study of the distribution of the virus in the blood and the organs yielded results similar to those obtained with several pathogenic bacteria.

The generalization of virus adsorbed on Al $(OH)_3$ was slower and less pronounced, and this fact probably contributes to the efficacy of the virus-Al$(OH)_3$ suspensions as vaccines.

The mechanism of infection in foot-and-mouth disease is more difficult to follow on account of the rapidity with which the disease develops in guinea-pigs, and we have not as yet obtained a clear impression of the different stages of the development of the disease. One striking feature which has been recorded by the British Commission is that in the early stages of the disease the virus can often be demonstrated in the blood, but not in the liver and the spleen. After a subcutaneous inoculation of a suitable dose of virus adsorbed on alumina, the generalization of the virus is greatly retarded and the virus is inactivated *in situ* within a few hours. If, after the inactivation is completed, the site of inoculation is excised, ground up, suspended in a fluid and re-injected in new guinea-pigs, the latter are actively immunized.

Prof. R. Doerr (Basel).

Im Gegensatze zu den vorliegenden Angaben kann man durch intravenöse Injektion bestimmter Stämme von Herpesvirus mit grosser Regelmässigkeit eine Infektion des Zentralnervensystems erzeugen und zwar eine primäre Myelitis des Lendenmarkes (Virusnachweis, Paraplegien der hinteren Extremitäten). Die Infektion des Lendenmarkes erfolgt direkt vom Blut aus, *nicht* durch Umleitung über die Peripherie. Verwendet man "nicht encephalitogene" Herpesstämme, so entsteht keine manifeste Myelitis, sondern eine *regionäre*, d.h. auf das Rückenmark beschränkte, nicht auf das Gehirn übergreifende Immunität. Durch intracerebrale Injektion geeigneter Herpesstämme konnte ebenfalls eine primäre Myelitis im Lendenmark hervorgerufen werden, eine Tatsache, welche Zweifel erwecken muss, ob der Mechanismus der Poliomyelitisinfektion den Annahmen von Hurst, Brodie, Faber u.a. entspricht. Es muss überhaupt betont werden, dass die Hypothesen über die Wanderung der neurotropen Virusarten in peripheren Nerven und ihre Ausbreitung im Zentralnervensystem, obwohl sie auf Experimente basiert sind, mannigfache Widersprüche aufweisen, welche einer synthetischen Erfassung des Gesamtproblems im Wege stehen.

Dr. J. T. Edwards (Pirbright, Surrey) also took part in the Discussion.

DISCUSSION *on* EVIDENCE CONCERNING THE AGENCY OF VIRUSES IN THE ÆTIOLOGY OF NEW GROWTHS

Chairman: Dr. Peyton Rous (New York).

Dr. Peyton Rous (New York), **Opener**: *Present Knowledge concerning Viruses as a Cause of Tumours.*

Tumours are not infectious under natural conditions. To cause them an extraneous agent would have to possess remarkable properties. When we consider the viruses in this connection we deal with agents whose potentialities are only partially apprehended.

It is known that viruses produce disease through an intimate association with tissue cells; and of such cells tumours are made. It is also known that some viruses cause cells to multiply; and through cell multiplication tumours arise. Cells, not the animal body, are the true hosts of the viruses, fostering and even protecting them. These facts bring the possibility near that an association of viruses with cells is responsible for some of the neoplastic diseases; and actually a tumour of the frog and numerous tumours of the domestic fowl have proved to be due to viruses. The growths of the fowl are typical neoplasms in their appearance and behaviour, and in the no less important respect that they are uninfective under natural conditions and sporadic in incidence. Where and how the causative viruses exist when not producing them is problematic, but it is known of some other viruses that they reside in the body and only exceptionally cause disease.

Evidence that viruses can give rise to tumours in mammals is not wanting. The wild rabbits in the mid-west of North America frequently carry on their skin papillomas which are caused by a virus; and these growths, though manifestly infectious in ætiology and incidence, exhibit the immediate traits characterising tumours as a class. In domestic rabbits the virus produces notably vigorous papillomas, and these frequently become true, metastasizing cancers. The discovery that a virus can cause a tissue disturbance out of which cancer arises has little importance in itself, for many, highly various, agents are carcinogenic in this way. But the rabbit virus produces papillomas that are themselves often on the brink of malignancy, that resemble the papillomas of undetermined cause occurring in other species, and that undergo the same changes as do these latter when they become cancerous. Numerous facts attest to an influence of the rabbit virus on the cancers. It does more than serve as their primary cause, it determines their time of occurrence, their character and, to no inconsiderable extent, their form. Yet neither this virus nor one antigenically related to it is responsible for the other rabbit tumours thus far tested;

and the eventual cancerous change is not merely an enhanced, virus-induced papillomatosis, but the expression of some further alteration. This can be greatly hastened, yet under natural conditions it is preceded by a "pre-cancerous" period like that observed in carcinomas generally. In interpreting these facts one must remember that the virus-induced papillomas and the cancers deriving from them are alike the outcome of the interaction of two elements, namely, the virus and the epidermal cell that it affects. After the two have become associated, either may alter or be altered secondarily, thus influencing the other and the character of the neoplastic process in due course.

Viruses are the most direct causes of tumours that are now known and the only agencies that bring them about, continue to actuate them and determine their character and course. One can readily explain all tumour phenomena in terms of what is known of viruses. Yet it does not follow that the generality of tumours are actually caused by these agents.

Dr. Ch. Oberling and **Dr. M. Guérin** (Paris): *La production de tumeurs avec l'agent de la leucémie transmissible des poules.*

La coexistence de leucémie et de tumeurs malignes chez la poule avait été notée par plusieurs auteurs et par nous mêmes, mais ce furent toujours des faits isolés où l'éventualité d'un hasard ne pouvait être exclue. Les recherches systématiques sur les rapports entre leucémie et tumeurs dont nous avons publié les premiers résultats en mars 1933 ont montré que notre souche leucémique donnait des tumeurs si l'on inocule, dans le muscle pectoral, du sang ou des fragments d'organes leucémiques (foie, moëlle osseuse) conservés à la glycérine et à la glacière.

Nous avons obtenu ainsi 26 tumeurs, en général de type sarcomateux, dont 7 avec métastases.

Nous avons réalisé le passage leucémie-tumeur et tumeur-leucémie à de multiples reprises. Le pouvoir hémotrope a persisté dans une tumeur après 8 mois d'évolution, et le pouvoir histiotrope s'est manifesté de nouveau après 40 passages leucémiques L'unicité de l'agent n'est donc pas en doute et on peut admettre alors que le virus leucémique a une double affinité cytotrope : hémotrope et histiotrope. L'affinité hémotrope est la plus marquée et elle se manifeste surtout si l'on pratique régulièrement les transmissions en inoculant du sang par voie intra-veineuse.

Le pouvoir histiotrope ne se manifeste que dans des conditions déterminées ; la simple atténuation du virus par un séjour à la glacière et à la glycérine ne suffit guère. Il importe : (*a*) d'utiliser des poules adultes, (*b*) d'utiliser pour les inoculations, non seulement du sang, mais des fragments d'organes (foie, moëlle osseuse), (*c*) de changer de mode d'inoculation, de préférence en passant de l'inoculation intra-veineuse, poursuivie pendant plusieurs passages, à l'inoculation intra-musculaire.

Ces faits semblent indiquer que le changement de l'affinité cytotrope du virus leucémique répond à un processus de sélection plutôt qu'à une atténuation comme nous l'avions pensé autrefois.

Dr. J. Furth (New York): *Viruses in the Ætiology of New Growths.*

The discussion is based on studies of neoplasms (leukosis and tumours) in mice and chickens. Chicken leukosis and tumours are produced by viruses; the known strains of mouse leukæmia and tumours, according to the available evidence, are not.

There are numerous viruses that produce in chickens either sarcoma or leukosis or both. Accordingly, they may be classified as simple and complex oncogenic viruses. The six different viruses isolated in our laboratories retained their characteristics throughout varied experimental conditions during a period of two to seven years. They range from some which yield free virus in abundance to one in which the presence of virus can be shown only with difficulty. Evidence for the existence of transmissible virus-free chicken tumours is wanting.

It is probable that viruses mutate, but this has yet to be proved. The transmissibility or virus ætiology of chicken carcinoma awaits further evidence.

The existence of complex viruses is indicated by tissue culture studies. Our chicken sarcoma 13 retained its ability to elaborate a virus causing leukosis and sarcoma after 122 days *in vitro*, while simple leukosis viruses perish *in vitro* within a few weeks in the presence of sarcoma cells or fibroblasts of blood-forming tissues. Multiplication of the viruses studied was obtained *in vitro* only in the presence of living cells that they have stimulated to neoplastic growth.

Many neoplasms of mammals are probably not produced by a virus, e.g., those which arise in X-rayed ovaries of mice one-half to one and one-half years after irradiation, and which can be traced to the regenerative changes that follow X-ray injuries; or the breast tumours that are dependent on ovarian hormonal activity.

Dr. E. Uhl, Dr. J. Engelbreth-Holm and **Dr. A. Rothe Meyer** (Copenhagen): *Production of Specific Antibodies against the Agent of Chicken Leukosis.*

Neutralization experiments with duck serum and leukotic plasma or leukotic chicken blood show that:

(1) the serum from normal untreated ducks does not neutralize the leukotic agent in chicken blood;

(2) the blood-serum of ducks immunized with leukotic chicken blood contains "antibodies" against the leukotic agent present in chicken blood;

(3) the blood-serum of ducks immunized with normal chicken blood does not neutralize the agent of chicken leukosis;

(4) these "antibodies" not only neutralize the leukotic agent present in cell-free plasma but have also a pronounced inhibitory effect on that associated with cells from diseased chickens.

Prof. G. P. Berry, Dr. J. A. Lichty and **Miss Helen M. Dedrick** (Rochester, N.Y., U.S.A.) *On the Relationship of Rabbit Fibroma (Shope) to Infectious Myxomatosis (Sanarelli), with a Method for changing Fibroma Virus into Myxoma Virus.*

Shope's observation that rabbit fibroma and infectious myxomatosis are related immunologically has been confirmed and extended. We have found that infection with either fibroma or myxoma virus induces in domestic (*Oryctolagus*) and cottontail (*Sylvilagus*) rabbits a resistant state to either virus, this state being characterized by the development in their serum of neutralizing capacities for either virus. The elaboration of a sensitive neutralization technique and the establishment of myxomatosis for the first time in the cottontail, made these results possible. In the cottontail myxoma virus seldom kills, even after 30 serial passages.

Fibroma virus was changed into myxoma virus by a method suggested by Griffith's studies on the transformation of pneumococcal types, i.e., rabbits inoculated with mixtures of fibroma virus and heat-inactivated myxoma virus developed myxomatosis. Inactivation was accomplished by heating for 30 minutes at temperatures of 60°–90° C. Transformation has occurred regularly with 60°–75° C. material, but not with 90° C. material. It has occurred in both domestic and cottontail rabbits, and with different strains of fibroma and myxoma virus. Test rabbits developed fatal myxomatosis, with characteristic histopathology. From them typical myxoma virus was readily obtained. The completeness of inactivation has been adequately controlled. Transformation has followed the use of heat-inactivated myxoma material only; other viruses, gastric mucin, suspensions of various tumours, etc., have been ineffective.

These experiments support the hypothesis that fibroma virus and myxoma virus are different strains of one basic virus. Something in myxomatous material, thermostable up to 75° C. and by itself unable to induce myxomatosis in rabbits, changes fibroma virus into myxoma virus. Its action may resemble that of bacterial haptens. It is suggested that experiments similar to these may prove useful in exploring the natures of other viruses and their relationships, and possibly their rôle in the causation of tumours.

Dr. P. R. Peacock (Glasgow): *A Comparative Study of Filterable and Non-filterable Fowl Tumours.*

The filterable tumours studied were the Fujinami Sarcoma and McIntosh Sarcoma No. 5. The non-filterable tumours were eleven transplantable strains of sarcoma induced by intramuscular injection of tar or dibenzanthracene in various solvents. All fowls injected with filterable tumours have been kept in the Research Department of the Glasgow Royal Cancer Hospital and those injected with induced tumours at the Farm Laboratory, five miles distant from the Hospital, the only common factor between the two laboratories being the personal one.

No case of filterable tumour has occurred at the Farm Laboratory during five years, so that the precautions taken against chance contamination appear to have been effective.

The following methods of study have been employed :—

(1) Experimental transmission by grafts and cell suspensions, as well as by filtrates or desiccates for each tumour strain;

(2) Histological studies of the filterable and non-filterable tumours;

(3) Radium treatment of McIntosh No. 5 sarcoma (filterable), and of G.R.C.H.13 sarcoma (non-filterable).

It appears that the induced fowl tumours of the G.R.C.H. series show no evidence of the presence of a cell-free ætiological agent; induced tumours failed to resist desiccation and filtration; radium treatment, which cured transplanted G.R.C.H./13 sarcoma, failed to prevent the spread of McIntosh 5 sarcoma, though locally effective.

Filterable and non-filterable tumours could not be distinguished histologically, though each strain possesses histological characteristics, which remain true to type through serial passages.

Filterable and non-filterable fowl sarcomata of similar histology probably exist, and contamination of a non-filterable tumour by a filterable agent might cause no recognizable change in the histology of the strain. This possibility must be borne in mind in assessing the ætiological rôle of cell-free agents derived from induced tumours after many passages as non-filterable tumours.

Prof. J. McIntosh (London): *Virus Infections in Tar-induced Tumours (Sarcomata) of the Fowl.*

Observations made during the last four years have shown that some tar-induced tumours of the fowl—just as the Rous sarcoma—can be transmitted by cell-free filtrates. Of six tar-induced tumours (Nos. 2, 3, 4, 5, 7 and 9), which have been successfully passaged, three (Nos. 2, 3 and 5) could be transmitted after a few passages by cell-free filtrates.

The successful filtration of these tumours appears to be largely a question of virulence. Propagation is usually difficult at first as

only a few of the inoculations take after long intervals of time. After a series of passages the percentage of takes increases and growth is more rapid, and at this stage filtration is more apt to be successful. There are two slow-growing tumours (Nos. 7 and 9) which still resist filtration. These observations remind one of the *non-filterable stage* of the Rous tumour No. 1.

Biochemical investigations have failed to show any constant difference in the metabolism of the filterable and non-filterable tar-induced tumours. Immunity experiments show that the viruses in the filtrates of these tar tumours possess antigenic relationship to themselves and to the Rous tumour.

Further evidence of the virus nature of the infecting agent in these tumours has been adduced by the sedimentation of the viruses by means of a high speed centrifuge giving a gravimetric pull of about 100,000 times that of gravity. By this procedure the supernatant fluid can be rendered non-infective while the deposit contains the infective material.

Dr. C. H. Andrewes (London) : *Viruses in Non-filterable Tumours.*

Few will deny that the essential agent in the ætiology of filterable neoplasms is a virus. It can be shown that a virus is also concerned in, at any rate, some non-filterable tumours.

(1) Rous sarcoma No. 1 and Mill Hill 2 fowl endothelioma pass through phases in which, for many months, all attempts at filtration fail. Yet the virus has not disappeared, for filterability is ultimately regained.

(2) The Shope rabbit papilloma is filterable in its earlier stages ; but later, when the growth has become frankly malignant, propagation by means of cell-free material fails. But continued presence of the virus can be demonstrated indirectly ; for Rous and his co-workers have shown that rabbits in which grafts of the now unfilterable tumour grow, develop in their sera antibodies which neutralize the papilloma virus.

(3) Like Peacock and others, I have failed to filter fowl sarcomata induced by tar and dibenzanthracene. But I have been able with one such fowl tumour (the Sheffield tar sarcoma of Mellanby) to show by indirect means that a virus is present. Cells from this tumour inoculated into pheasants grow for 1-2 weeks, sometimes longer ; ultimately regression always occurs. Such pheasants develop antibodies which neutralize Rous sarcoma virus. (I have shown previously that viruses of histologically distinct tumours may be closely related serologically.) It can be demonstrated that this neutralization is not caused by anti-fowl properties in the sera. It is deduced that this non-filterable fowl tar sarcoma contains a virus, closely related serologically to Rous sarcoma virus, but not directly demonstrable by filtration of the tumour. Possibly some tumour-viruses are unable to infect normal cells.

Dr. W. E. Gye and **Mr. L. Foulds** (London) : *Anti-sera, which neutralize the Virus of the Rous-Sarcoma No. 1, prepared by injecting Rabbits with Cell-free Extracts of a Non-filterable Dibenzanthracene Tumour of Fowls.*

A fibro-sarcoma of the fowl, originally induced by injections of dibenzanthracene, has been tested again and again for non-cellular transmissibility during the past three years, and always with negative results. It has not been possible to transmit the tumour with centrifuged extracts. Rabbits have been immunized by injecting emulsions of tumour cells, others by injecting spun extracts, and others again with Berkefeld filtrates of the tumour. The inactivated sera of all these rabbits have been proved to neutralize cell-free extracts of the Rous tumour No. 1 ; they fail to neutralize extracts of Fujinami's myxosarcoma.

Dr. W. E. Gye and **Mr. W. J. Purdy** (London) : *Neutralizing Sera produced by Injections of Extracts of Normal Fowl Tissues.*

The successful preparation of neutralizing sera by injection of extracts of fowl tumours into alien species of animals is often met by the criticism that the neutralizing antibody is formed as a response to an antigen which is intrinsically " fowl," and which may be present in normal tissues. We have immunized goats, horses, rabbits and ducks with extracts of normal tissues ; in most of our experiments chick embryo has been used, but in some we have used adult tissues. The sera obtained are inactivated by heating at 55° to 56° C. for 30 minutes ; they then bave no neutralizing power. When fresh guinea-pig serum is added to the mixture of immune serum and tumour extract, almost complete neutralization of the tumour agent occurs. This neutralizing antibody is readily absorbed by emulsions of normal fowl cells, whilst the neutralizing antibody formed in response to injections of extracts of tumours is unaffected by absorption with normal tissues. Extracts of Fujinami's tumour infect both ducks and fowls ; antibodies to extracts of Fujinami's tumour, whether from duck or fowl, neutralize virus taken from either duck or fowl. Antibodies to normal fowl cells neutralize, in the presence of complement, extracts of fowl-grown Fujinami tumour, but not of duck-grown tumour ; the opposite occurs with antibodies to normal duck cells.

Dr. C. Russell Amies (London) : *The Particulate Nature of the Agents of Rous Sarcoma No. 1 and of the Fujinami Myxosarcoma.*

Ledingham and Gye have recently reported that the tumour-exciting agents of the Rous No. 1 sarcoma and the Fujinami myxosarcoma can be deposited from cell-free tumour extracts by means of the high-speed centrifuge. A long series of quantitative experiments has shown that a centrifugal force of about 20,000 times the force

of gravity when applied for one hour will completely remove the agent from a highly active cell-free tumour extract, and that the agent can be recovered quantitatively from the deposit so obtained.

By means of repeated fractional centrifugation it is possible to obtain suspensions devoid of fowl protein which are actively tumour-producing in a dilution of 1 in 1,000. These pure suspensions of the tumour agent are unaffected by hyper-immune rabbit anti-fowl sera *in vitro*, whereas the addition of the serum taken from a tumour-bearing fowl inhibits their activity irrespective of whether complement is added to the mixture before inoculation or not.

Microscopical examination of actively tumour-exciting suspensions free from fowl protein by the method of dark-ground illumination reveals the presence of numberless particles of slightly varying size. These particles are below the limit of resolution with visible light, but their constant presence in infective suspensions, their absence in the non-infective supernatant fluids, and the knowledge already available regarding similar particles (elementary bodies) in virus diseases leads almost inevitably to the conclusion that these bodies represent the actual causal agent.

The fact that these "elementary bodies" are agglutinated in specific fashion by the serum of fowls bearing actively growing or regressing tumours has also been confirmed. Agglutinins, however, are often not demonstrable and have seldom been obtained in high titre.

Prof. P. Uhlenhuth (Freiburg i.B.).

Mit seinen Feststellungen im Tierexperiment und seiner Virustheorie ist durch M. H. Gordon in der Frage des Hodgkin ein völlig neuer Gesichtspunkt entstanden. Bei umfangreichen Nachprüfungen konnte ich mit meinem Mitarbeiter Dr. Wurm in bis jetzt 5 Fällen von Lymphogranulomatose in den Drüsen dieses von Gordon gefundene Agens nachweisen, das bei Kaninchen und Meerschweinchen in das Zentralnervensystem eingebracht, ein charakteristisches, durch Spasmen und später Paresen ausgezeichnetes Krankheitsbild hervorruft. Verimpfungen andersartig erkrankter Drüsen hatten diese Wirkung im Tierversuch nicht. Somit hat der tierexperimentelle Nachweis dieses Agens nach den bisherigen Untersuchungen diagnostische Bedeutung, ganz besonders in den Fällen, wo die histologische Untersuchung eine eindeutige Diagnose nicht zulässt. Seiner Natur nach scheint dieses Agens invisibel zu sein, ist durch bakteriendichte Filter passierbar und gegen Hitze ziemlich resistent. Wie manche Virusarten besitzt es gegenüber Glyzerin, Phenol und Äther beträchtliche Resistenz. Der Nachweis von Elementarkörperchen ist uns bis jetzt noch nicht gelungen. Auch war es bis jetzt nicht möglich beim Kaninchen irgendwelche Immunität zu erzielen. Rekonvaleszenten blieben gegenüber einer nochmaligen intracerebralen Impfung weiterhin empfänglich. Endlich war es uns bis jetzt nicht möglich, dieses Agens zur Vermehrung zu bringen,

weder in Tierpassagen noch in Gewebekulturen. Die Frage, ob es sich dabei um ein Toxin, Enzym oder Virus handelt ist noch offen. Die Bedeutung dieses Agens bezüglich der Ätiologie des Hodgkin ist noch unklar. Bei seiner scheinbaren Spezifität scheint aber ein innerer Zusammenhang mit der Noxe des Lymphogranuloms wohl sicher.

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Dr. T. M. Rivers (New York).

The observations of Drs. Andrewes and Gye are not conclusive evidence that a virus is the cause of their non-filterable tumours. Reasoning in the manner used by them, one might say that virus III induces in rabbits the tumour described by Brown and Pearce because the tumour-bearing rabbits are immune to virus III and possess neutralizing antibodies in their serum. The tumour carries the virus, but as shown by experiments is not caused by it.

Prof. C. H. Browning (Glasgow).

An illustration is afforded from my own work that malignant tumours arise from cells previously altered in some way as mentioned by Dr. Rous, viz., following the subcutaneous injection in mice of a water-soluble benzoyl-amino-styryl dye which is precipitated in fine granules by the lymph proteins. These insoluble granules are taken up by the cells of a phagocytic tissue which is formed, and from this tissue a sarcoma develops frequently.

Dr. H. J. Fuchs (London).

Die Möglichkeit, durch Behandlung von gesunden Tieren mit dem Serum von Tumorträgern, deren Tumoren nicht-filtrierbar sind, ein Antikörperserum zu erhalten, das das Angehen dieser Tumoren mehr oder weniger verhindert, findet eine Analogie in den Anatoxinen, Anavenins und den Versuchen von W. M. Stanley mit dem kristallisierten Protein, welches die Mosaikkrankheit erzeugt: alle diese, durch spontane Abschwächung oder vorsichtige chemische Behandlung nicht mehr krankheitserzeugenden Antigene erzeugen die gleichen Antikörper wie die aktiven, krankheitserzeugenden. Damit findet auch die Schwierigkeit bei Säugetiertumoren filtrierbare, zellfreie, aktive Tumorextrakte zu erhalten eine Erklärung. Durch Verwendung flüssiger Luft und starker Salzlösungen ist es in einem Vorversuch gelungen, aus dem Brown-Pearce-Carcinom zellfreies Tumorextrakt zu gewinnen und nach mehrmaliger Umkristallisierung—ähnlich der Methode W. M. Stanleys—eine kristalline Substanz zu erhalten, die bei 11 Kaninchen von 12 gleicher Rasse der Tumorträger, von denen das Ausgangsmaterial stammte, einen histologisch gleichartigen Tumor nach Injektion in den Hoden erzeugte.

Dr. C. A. Mawson (London).

With the aid of a centrifuge developing 15,000 r.p.m. it has been possible to obtain complete sedimentation of the active agent of the Rous sarcoma No. 1. By a process of fractional centrifugation similar to that used by Dr. Amies, preparations of minute bodies of uniform size have been made which were highly active in transmitting the sarcoma. However, preparations having exactly similar optical properties have been obtained by the same technique from normal tissue and from glycogen solution. The apparently uniform suspensions of "elementary bodies" obtained from Rous sarcoma may thus consist partly of inert material similar to that which arises from normal tissue. The particular technique used ensures that the particles in the final product shall be of a uniform size, and this goes far towards explaining their uniformity of appearance under the dark-ground microscope. Contamination of suspension of "elementary bodies" with inert material would account for the fact that little correlation exists between the number of bodies observed and the potency of preparations. Elution of the Rous agent adsorbed on alumina C has given preparations which contained comparatively few bodies and which were as potent as concentrates obtained by centrifugation and containing enormous numbers of these particles.

Dr. W. E. Gye (London), **Dr. C. H. Andrewes** (London), **Dr. P. R. Peacock** (Glasgow), **Prof. J. McIntosh** (London), **Dr. C. Russell Amies** (London), **Dr. Peyton Rous** (New York) and **Dr. Homer F. Swift** (New York) also took part in the Discussion.

DISCUSSION *on* MECHANISM OF IMMUNITY IN VIRUS DISEASES AND PRACTICAL APPLICATIONS THEREOF

Chairmen: Prof. J. C. G. Ledingham (London) and **Sir Patrick Laidlaw** (London).

Prof. J. C. G. Ledingham (London), **Opener.**

Perhaps the most important practical problem with which virus workers are faced to-day is that of securing safe and reasonably efficient methods of prophylaxis for the more serious types of virus disease in man and animals. Jenner's experimental demonstration of the capacity of cowpox to confer resistance to smallpox remains, with certain reservations, the touchstone by comparison with which all subsequent efforts in this sphere must be judged. It is probable, in fact, that present-day efforts to ensure the complete safety of immunization procedures by the use of truly killed virus agents, would be less urgent if it were found feasible by some happy chance

to light on fixed, innocuous, and antigenically-efficient variants of the type of cowpox or variola-vaccine that would give protection against the major diseases from which they might be directly derived or with which they might, perhaps by accident, be found to be related. Consideration of all the recent work on this subject points to the conclusion that genuinely killed virus agents, whatever the lethal agent employed, confer only the most trivial and ephemeral protection, though even such may be useful as a basis for subsequent safe reinforcement by the living virus. Interest is now rightly concentrated on the study of the elementary bodies, admittedly the sole effective agents in all virus diseases, and the opportunity is now offered for the investigation of their antigenic constitution. Apart from Craigie's work on the L and S agglutinogens of the Paschen bodies, we have not yet such knowledge as will enable us so to kill them that their prophylactic antigen, if such exists, remains intact. It is desirable that enquiries in this direction be prosecuted intensely with the purest suspensions of these bodies and here it should be remembered that the resistance-producing properties of killed bacteria are now only becoming more thoroughly understood, as we learn more of their constituent antigens. With regard to formalin treatment, for example, Felix has shown that formolized *Vi* strains of *B. typhosum* stimulate the production of abundant agglutinins for *Vi* strains, but only an insignificant amount of protective antibodies, whereas treatment with alcohol produces both types of antibody in amounts comparable with those produced by the living *Vi* organisms. We have here, perhaps, a useful line for further work, for beyond question it should be our ultimate objective to secure protection, at any rate in man, with both safe and efficient reagents.

The antiviral substance: Discussion is invited on the probable mechanism of action *in vivo* of the neutralizing or antiviral body elaborated in the course of, or in convalescence from, virus disease. It is a problem which perhaps can be solved only by direct observation of the behaviour of the elementary bodies in the living animal or in tissue culture. The antigen that gives rise to the antiviral body may, in fact, be very different from that which stimulates the production of agglutinins and complement-fixing antibodies. Another problem in this field is the source of origin of the antiviral body in persons not known to have passed through clinical attacks and the explanation of its persistence in the serum, years after infection. Our information is as yet scanty on one vital point, viz., the persistence of virus in the body after clinical recovery.

Other antibodies, particularly agglutinins for elementary bodies: It has now been quite definitely established that agglutinins occur in the sera of virus-infected animals for the corresponding elementary bodies present in concentrated pure suspensions (vaccinia, variola, fowlpox, varicella, zoster and the filterable fowl tumours). A method is now available of differentiating serologically (by agglu-

tination and agglutinin-absorption) clinically related virus agents (e.g., the pock diseases, herpes and congeners, Shope fibroma and myxomatosis, influenza and the common cold, etc., etc.). With the gravitational fields now at our disposal for the deposition of even the minutest virus agents, I see no serious technical difficulties in the way. My own recent work in this latter field, and still in progress, has been concerned with the serological relationship of Myxoma to the Shope Fibroma, a study commenced before Berry and Dedrick's remarkable findings. Working with pure suspensions of the elementary bodies of myxoma and fibroma, I find that rabbits inoculated with fibroma may quickly develop agglutinins for myxoma but often little or none for fibroma until a dose of myxoma is given, in response to which, agglutinins both for myxoma and fibroma appear early in high titre. Some few absorption experiments so far carried out, show that when a serum containing agglutinins both for myxoma and fibroma is absorbed either with fibroma or myxoma elementary bodies, the fibroma agglutinins are completely, and the myxoma agglutinins perhaps less completely, removed. It would be of great interest if the fibroma virus could be proved to contain a "rough but *Vi*-containing" variant of the full "smooth *Vi*-containing" myxoma antigen.

Dr. T. M. Rivers (New York): *Intradermal Vaccination against Smallpox by means of Culture Vaccine Virus.*

Diluted culture virus, 0·1 c.c., is injected into the skin of the upper arm or thigh by means of a tuberculin syringe in a manner similar to that used in making an intradermal tuberculin or Schick test. After the injection has been completed it is essential to cleanse the needle wound thoroughly with alcohol in order to prevent the formation of a vesicle on the surface of the skin. No dressing or special care is indicated, i.e., the arm or thigh can be freely bathed throughout the evolution of the intradermal lesion.

When an individual who has never had a successful vaccination receives the culture virus intradermally, nothing abnormal is noted at the point of inoculation until 4 to 9 days later. Then a small red papule appears which gradually becomes larger until the area of induration and erythema is 2 or 3 cm. in diameter. At the height of the reaction, which is usually 4 to 6 days after its initiation, a secondary zone of erythema, less intense than the primary, makes its appearance around the lesion, remains for a day or two, and then rapidly disappears. The induration gradually becomes less marked and as a rule is no longer discernible 3 weeks after the appearance of the lesion. If the inoculation is made properly there is no vesicle formation to leave a scar.

The earliest revaccination reaction to occur appears within 6 hours after inoculation, while others do not become evident until 2 or 3 days later. Those that appear rapidly, consist of small red papules accompanied by itching and disappear in a day or two.

The ones that develop more slowly, are larger than the others, endure for 3 or 4 days, and gradually disappear to be followed by a slight desquamation of the involved skin.

Dr. J. Craigie (Toronto): *The Influence of Inactivation on the Antigenic Qualities of the Elementary Bodies of Vaccinia in the Rabbit.*

The following hypothesis may explain the loss of immunizing qualities which accompanies inactivation of vaccinia virus. Immunity to this virus is produced by labile antigens which are usually inactivated by treatment which renders the virus non-infective. Even if sufficient immunizing antigens survive inactivation of the virus the resultant immunity is of short duration, since no living virus is introduced which, surviving in cells, can prolong the immunity.

This hypothesis was tested by a study of the antigenic qualities of elementary bodies inactivated in various ways. These antigenic qualities comprise the production of L and S antibodies (agglutinin and precipitin), the production of neutralizing and viricidal antibodies, and the modification of response of the rabbit to subsequent inoculation of infective elementary bodies. Neither the L nor the S antibodies appear to play a major rôle in immunity or in the neutralizing and viricidal reactions. Immunity to vaccinia virus is usually estimated by resistance to dermal or intradermal infection in terms of minimal infective doses of virus. A more satisfactory method after injection of inactivated elementary bodies in the rabbit is to inoculate virus by scarification three to five days later and to compare the results with those on controls. Some suspensions of elementary bodies inactivated with formalin or alcohol can produce an accelerated vaccinial reaction comparable to that produced by infective elementary bodies. Since survival of a few elementary bodies in these preparations can be excluded, the presence of antigens other than L and S is possible. By postulating a third antigen, one may explain this vaccinial reaction in two ways: (1) this antigen is inactivated to a variable degree concomitantly with the elementary bodies; (2) it is associated with actively multiplying elementary bodies in infected cells and its amount varies in washed suspensions of these bodies. Agglutinin absorption tests also point to the existence of this third antigen.

Mr. R. E. Glover (Cambridge): *The Dermotropic Viruses of Animals. Immunological Relationship of Viruses of Variola-Vaccinia Group.*

Recent experiments by Nelson have shown that sows inoculated with vaccinia prior to farrowing transmit protective substances to their offspring by way of the colostrum. In the contagious

pustular dermatitis of the sheep, which is regarded as closely allied to the variola-vaccinia group, a dermal eruption induces a very solid immunity; moreover, the blood serum contains protective antibodies. Nevertheless, lambs born of ewes vaccinated during pregnancy are almost fully susceptible to the disease, i.e., there is no transference of immunity either by the placenta or the colostrum.

The most satisfactory method of determining the relationship of the various members of the variola-vaccinia viruses is by cross-immunity tests. They may be conveniently divided into two groups. The first comprises the true variola of man, the sheep, the pig and the fowl, which are generally regarded as entirely separate entities. They are strongly species specific and immunologically distinct from one another. The second consists of vaccinia only, which is capable of affecting most species either naturally, as is the case in the horse, cattle and pig, or artificially, as in the sheep, goat and fowl.

There is still a third group, viz., the infectious dermatitis of the sheep and that of the goat. These conditions, although they are immunologically distinct from vaccinia and variola, yet are clinically similar to these diseases and produce experimental lesions which closely resemble those of vaccinia. In the sheep, dermatitis can be definitely differentiated from sheep-pox, but a clear distinction between dermatitis of the goat and goat-pox has yet to be furnished.

Dr. R. N. Salaman (Cambridge): *Immunity to Virus Diseases in Plants.*

In plants, attempts to demonstrate humoral immunity against fungi, bacteria and viruses, or to transfer by graft the immunity of one species to another, fail.

Against virus diseases there exists real and apparent immunity. There are two types of the latter: (1) after initial reaction the plant displays no further symptom; (2) "carriers" which show no reaction.

Thung has recorded the resistance of a tobacco plant suffering from mosaic to inoculation with virulent virus. Of the author's three strains of "X" virus, one, "X^G," is practically symptomless on tobacco and potato. Infection with "X^G" protects against infection with more virulent strains. Of two further sub-varieties one, "X^N," is intensely virulent for potato and tobacco, but "X^G" protects against it completely. Similar protection to sub-varieties of cucumber and tobacco mosaic have since been recorded.

Two facts emerge: (1) protection is specific to a particular virus group; (2) the plant contains the protecting strain, presumably in every cell. Strictly local invasions by the virulent strain occasionally occur in protected plants: in such spots the non-virulent strain probably failed to penetrate. The immunity conferred is therefore cellular.

Immunity apparently depends on the existence of non-virulent sub-varieties of the invading virus. There is no satisfactory evidence of multiple strains of potato virus "Y." A variety of "Y" of lower virulence, recently isolated, confers immunity against virulent strains.

Natural immunity to virus infection is local or general; in the former, spread of virus is inhibited by necrotic reaction at point of entry. Such immunity can sometimes be overcome by inducing rapid growth when foci develop in the fresh shoots and become themselves localized by necrosis. In natural immunity apparently, the virus cannot obtain lodgment in the cells. There is no evidence of antibodies in juices of such plants.

In green vein-banding associated with the "Y" or "X" viruses, if more than one strain of "X" virus be present, that of lower virulence is concentrated in the green band. With "Y" infection, the central portions of the green band are free from virus.

Dr. Wilson Smith (London): *Immunological Observations on Experimental Influenza.*

Influenza in the ferret results in a condition of complete resistance against reinfection lasting for a few months. Thereafter the immunity slowly wanes, but some virus-neutralizing antibodies remain in the circulation long after the animal has again become susceptible. Vaccination, during the period of waning immunity, results in a sudden increase of circulating antibodies and a return to the state of complete resistance. Similar vaccination of normal ferrets induces antibody formation and some degree of resistance against the experimental disease, but fails to immunize completely.

Mice can be completely immunized by vaccination but success depends upon factors not, as yet, completely under control. The mass of virus injected, the number of immunizing doses and the time intervals between doses are most important. If an adequate quantity of virus is given it makes little difference whether it be living or killed with formalin. A very important factor is the animal species from which the virus is obtained. Thus mouse virus has proved a much better immunizing agent for mice than has ferret virus.

Mice may also be passively protected against infection by relatively large doses of immune horse serum. The serum exerts a beneficial effect even when given as late as 72 hours after infection.

In man, the little evidence there is at present available suggests that circulating antibodies are of great importance. Attempts to infect two human volunteers with ferret virus failed but both cases had neutralizing antibodies in the blood stream at the time of test. Three cases are known in which the disease was probably contracted from experimental ferrets. In one of these the antibody response was studied. Before infection no detectable antibodies were

present but following infection there was a gradual rise of antibodies until they attained a high titre.

Dr. Thomas Francis, Jr. (New York): *The Vaccination of Human Subjects with the Virus of Human Influenza.*

Strains of the virus of human influenza have been cultivated in artificial tissue culture medium for a considerable period of time. The active virus so cultivated has been used for the subcutaneous vaccination of human subjects. The development of neutralizing antibodies, the duration of the antibodies so induced and the possible significance of the procedure were discussed.

Dr. R. E. Shope (Princeton, U.S.A.): *Types of Immunity to Swine Influenza.*

Three types of immune reaction to swine influenza were discussed:

(1) That established by infection or vaccination with the swine influenza virus alone. Swine with this type of immunity, when inoculated intranasally with the swine influenza ætiological complex of virus and bacterium, inactivate or destroy the injected virus, remain clinically normal and exhibit no influenzal lesions at autopsy. The sera of these animals neutralize swine influenza virus.

(2) That established by infection with a mixture of human influenza virus and *H. influenzæ suis*. Swine with this type of immunity, when inoculated with the agents causing swine influenza, remain clinically normal, exhibit no influenzal lesions at autopsy and do not permit the establishment of either swine influenza virus or *H. influenzæ suis* in their respiratory tracts. The sera of these animals do not, however, neutralize swine influenza virus. Swine infected with human influenza virus alone, develop an incomplete immunity to swine influenza.

(3) That established by vaccination with living or heat-killed cultures of *H. influenzæ suis*. Swine with this type of immunity usually develop mild symptoms of swine influenza when inoculated with the swine influenza ætiological complex, and at autopsy they show a less extensive pneumonia than do control animals. The sera of these animals do not neutralize swine influenza virus, and the virus administered in testing for immunity establishes itself throughout the respiratory tracts. *H. influenzæ suis* may or may not be cultivated from the pneumonic lungs.

Dr. Alice Chenoweth, Dr. Arthur D. Waltz, Dr. Henry W. Scherp and **Dr. Joseph Stokes, Jr.** (Philadelphia): *Studies in Active Immunization with the Viruses of Human and Swine Influenza.*

Preliminary experiments performed in September and October, 1935, gave evidence of increased neutralizing properties in the

serum of individuals injected intramuscularly with the live viruses of human and swine influenza. The injections consisted of Berkefeld filtrates of 10 per cent. emulsions of mouse lungs containing the virus.

A study was then made in a large State colony consisting of approximately 800 boys and men. Approximately one-third of the individuals in each house in the colony were inoculated immediately before the appearance of an epidemic of influenza in the surrounding region. Men and boys were divided equally for injection, and approximately 125 were injected with each virus, or 250 individuals in all. A control group of approximately 550 remained. The results of this study were presented.

Prof. E. W. Schultz (Stanford Univ., U.S.A.): *Immunity to Poliomyelitis and the Problem of Prophylaxis.*

It has been established that poliomyelitis is caused by a highly neurotropic virus which normally enters the central nervous system by the olfactory nerve and travels to the anterior horn cells by axonal paths. Some virus escapes from infected nerve cells, and this may account for the appearance of specific antibodies in the blood. These antibodies, however, do not seem to play an important rôle in resistance to this disease; recent experimental work shows that monkeys cannot be effectively protected against intranasal instillation of virus even with large doses of a high titre immune serum. While vaccines may induce the formation of humoral antibodies, they do not appreciably modify the susceptibility of nerve cells. Naturally acquired active immunity to this disease appears to rest largely on a modification of susceptible nerve cells, the result of intimate contact with virus, and in its fundamental aspects does not seem to depend on the presence or absence of humoral antibodies. Inherited constitutional factors no doubt influence the ease with which resistance to this disease is acquired.

While the results of serum prophylaxis and of the application of vaccines do not argue clearly for their effectiveness in controlling this disease, recent observations indicate that temporary protection may be afforded by the previous treatment of the nasal mucosa with chemical agents which tend to diminish the permeability of the olfactory portal for this virus.

Dr. G. M. Findlay (London): *Immunization against Yellow Fever in Man.*

More than a thousand persons have now been immunized against yellow fever by injections of immune serum and living virus. During the past year the living virus employed has been a pantropic strain grown in chick embryo tissue and serum-Tyrode: this has

replaced the neurotropic virus passaged in mouse brains, the risk of producing yellow fever encephalitis being thus reduced to a minimum. The use of chick embryo tissue reduces the dangers inherent in the use of mouse embryo tissue which may be contaminated with other viruses pathogenic for man. After repeated subcultures, the pantropic virus becomes so attenuated that it only rarely kills rhesus monkeys ; it is, however, still fatal to hedgehogs and can easily be reactivated. Two types of yellow fever immune serum have been used : (i) human serum from persons artificially immunized or recovered from natural infections, (ii) serum from immunized horses. Although the immune bodies in the latter serum can be concentrated, serum reactions may occur in from 10 to 15 per cent. of those injected. Among more than 200 persons immunized with human immune serum and attenuated pantropic virus no serious reaction has occurred. The immune body titre rises to a maximum four to five weeks after inoculation, then gradually falls. Immune bodies can often be detected after more than two years and an injection of virus alone during this period will again raise the titre. Immunization with dead virus has been partially successful in mice, but not in hedgehogs and monkeys, owing to the enormous doses of virus required. Since a method of immunization has been developed, no case of yellow fever has occurred among those exposed to laboratory infections.

†**Prof. W. J. Tulloch** (Dundee) : *Notes on the Reaction of Tissues to Infection with Virus Agents.*

If we accept the virus agents as subvisible microbes, then we should expect that tissue response to invasion by them will not differ fundamentally from that called forth by bacterial infection.

The formation of new tissue akin to granuloma, which may in some instances resemble true neoplasm, is frequently a feature of infection with virus agents. On the other hand, among these agents there is none which is frankly pyogenic, although activity on the part of phagocytes has been observed in vaccinia.

As to humoral response, an overwhelming weight of evidence has accumulated to show that the virus agents act as antigens. Therefore antibodies exhibiting specific protective, complement-fixing and precipitating properties are called forth in animals immunized against these agents. However, little is to be expected from the employment of antiserum in therapeutics of virus diseases because of the peculiar invasive quality of the causal agents.

In contrast, the diagnostic value of such sera in serological tests may be considerable.

Owing to the difficulty of cultivating the agents, such tests should be designed to demonstrate the presence of virus in morbid material

† This paper was taken as read with the consent of the author, who was present.

rather than the presence of antibodies in serum. This latter method does not show that infection exists, but merely that it has existed at some time. When conducting such tests one should remember that the antigen is in a finely particulate state, because the precise technique most suitable for showing the formation of any "antibody-antigen complex" is conditioned by the degree of particulation of the reagents.

†**Prof. J. McIntosh** (London): *Antibody Production by Virus Antigens (Elementary Bodies).*

As there still seems to be some ambiguity about the mechanism of virus immunity, a number of observations have been made on the antigenic power of elementary bodies (pleuropneumonia, vaccinia, etc.). The antigens used were in each case pure suspensions of elementary bodies prepared by filtration and fractional centrifugalization.

Complement fixation reactions have given results comparable with those obtained with bacteria, but inconclusive results have so far been obtained in agglutinin reactions.

Dr. F. F. Tang (London): *Equine Encephalo-myelitis—Immunizing Value of Virus subjected to the Photodynamic Action of Methylene Blue.*

It is now known from the work of Clifton, Herzberg, Perdrau and Todd and others that bacteriophages and many viruses can be inactivated by the photodynamic action of methylene blue and certain acridine dyes. Perdrau and Todd reported that the virus of canine distemper inactivated by methylene blue in the presence of light preserved its antigenic properties and could be used to immunize ferrets. Galloway showed that 26 out of 31 rabbits, which had received more than one dose of rabies fixed virus rendered non-infective by the photodynamic action of methylene blue or proflavine, survived an intramuscular test dose of fresh virus which killed 15 out of 16 controls.

It has now been shown that the virus of equine encephalomyelitis can be rendered non-infective by the photodynamic action of methylene blue. The time necessary for inactivation varied with the original titre of the filtrate or unfiltered suspension subjected to irradiation. A broth filtrate having a titre of 10^{-6} to 5×10^{-6} was not inactivated in less than 30 minutes. An unfiltered broth suspension of glycerinated infective brain with methylene blue was rendered non-infective by irradiation for 20 minutes. An unfiltered broth suspension of fresh infective brain having about the

† This paper was taken as read with the consent of the author, who was present.

same titre was still found to be infective after 30 minutes of irradiation. The virus was inactivated more quickly in a saline than in a broth medium, but this could be explained by the lower titre of saline suspensions of virus.

Virus-dye mixtures irradiated for 3 hours had little or no immunizing value when tested on guinea-pigs. Virus-dye mixtures irradiated for 1 hour and proved to be non-infective, when inoculated intracerebrally or subcutaneously into guinea-pigs protected them against a subsequent test dose of virus inoculated into a peripheral site (intramuscular and intraperitoneal inoculation) but gave no protection against an intracerebral inoculation of fresh virus. Virus-dye mixtures irradiated for 30 minutes, which by all the tests available for detecting the presence of living virus (inoculation of the mixtures intracerebrally into guinea-pigs and mice, inoculation into chick embryo tissue cultures, inoculation into developing hen's and duck's eggs) did not appear to be infective, nevertheless protected guinea-pigs against both intracerebral and intramuscular test inoculations.

A single intracerebral inoculation of 0·25 c.c. of virus-dye mixtures irradiated for 30 or 60 minutes did not protect guinea-pigs against a subsequent intracerebral inoculation of fresh virus.

Virus-dye mixtures irradiated for 10 or 20 minutes, which proved to be infective when tested by intracerebral inoculation of guinea-pigs but did not infect when introduced subcutaneously, also protected guinea-pigs against both intracerebral and intramuscular inoculations.

Many hold the opinion that it is not possible to immunize the central nervous system against infection with a virus by employing the inactivated virus as a vaccine. If this view is correct then it must be concluded that the tests employed to detect the presence of small traces of unaltered virus in virus-dye mixtures in the present experiments were inadequate.

Dr. J. Laigret (Tunis).

M. Findlay a rapporté une statistique de mille séro-vaccinations antiamariles. Les Instituts Pasteur ont vacciné 15,000 volontaires, sans sérum ; cela permet d'affirmer que l'injection préalable de celui-ci n'est pas nécessaire.

Les réactions méningées, qu'on a observées avec l'un et l'autre procédé, sont des incidents très rares et toujours bénins. Leur cause est encore mal connue. On a incriminé le virus de Traub-Armstrong qui existe dans d'assez nombreux élevages ; il est pathogène pour les souris ; on peut donc l'éliminer en n'utilisant que des animaux en parfait état de santé.

Toutefois, nous avons isolé de nos souris, saines d'apparence, un virus qui n'est pas pathogène pour elles, mais qui l'est pour le cobaye et pour le singe. Ce même virus a été retrouvé dans le

liquide céphalo-rachidien d'un vacciné atteint de réaction meningée. Son ubiquité est très grande. Nous l'avons obtenu aussi du liquide céphalo-rachidien d'un non-vacciné ; il existe dans l'embryon de souris et dans l'embryon de poulet. S'il est la cause des réactions meningées, ce qui n'est pas démontré, on ne peut pas espérer que l'emploi des vaccins de culture supprimera ces accidents.

Dans les colonies françaises, on continue d'appliquer de plus en plus largement le vaccin préparé avec le virus de Theiler desséché et enrobé. Les 15,000 personnes traitées ont été protégées. Un vacciné, soumis à des piqûres de stégomyias infectés, qui ont tué deux singes de fièvre jaune, a supporté l'épreuve. Notre vaccin crée un pouvoir protecteur puissant dont le taux n'a pas baissé, chez les plus anciens vaccinés, après cinq ans. Le procédé est pratique, à la portée de tous les médecins ; le vaccin peut être expédié par la poste aérienne.

Étant donné ses avantages, j'estime qu'il faut, sans négliger l'étude de perfectionements nouveaux, continuer et encourager l'application de la méthode.

Dr. A. Walch-Sorgdrager (Amsterdam) : *Immunity in Mice against Yellow Fever.*

Prof. Schüffner and myself have studied immunity in mice against yellow fever. Our experience pointed to the fact that a mouse which survives infection with yellow fever virus develops only a feeble immunity or no immunity at all, since a number of such mice after a reinoculation of the virus died just like normal ones do after an injection of virus into the brain.

To study this point mice surviving a positive or negative protection test by the Theiler or Sawyer method, or an inoculation into the peritoneum with yellow fever virus with or without a previous injection of starch into the brain, were reinfected.

The following are our conclusions :—

1. To disclose immunity in mice after one previous injection of yellow fever virus the indirect (or peritoneal) method is superior to the direct (or cerebral) method, the latter giving only a small percentage of surviving mice. After two previous injections the direct method proves useful.

2. After reinfection by the indirect method a much larger percentage survived, when the first inoculation of virus had been made into the peritoneum, than when it had been made into the brain.

3. Mice which had received virus without protective serum seemed to develop a stronger immunity than those which had received virus + immune serum, the immunity in the first case involving even the brain.

Dr. Luis E. Hurtado (Guatemala, Central America): *The Influence of the Temperature of the Atmosphere on the Spread of Yellow Fever.*

In the 1919 epidemic of yellow fever in San Salvador the grave and fatal cases occurred especially in foreigners or in natives who dwell normally in a cold climate. When the infection spread along the Pacific coast the worst foci were in places which were affected by a heat wave at the time. This observation is the very opposite of what one finds in outbreaks of influenza. The virulence and spread of yellow fever may therefore be favoured by a high atmospheric temperature.

SECTION 3.

BACTERIA AND FUNGI IN RELATION TO DISEASE IN MAN, ANIMALS AND PLANTS

DISCUSSION *on* THE SIGNIFICANCE OF SEROLOGICAL AND CULTURAL TYPES OF BACTERIA AND FUNGI PATHOGENIC TO MAN, ANIMALS AND PLANTS IN RELATION TO EPIDEMIC, EPIZOOTIC AND EPIPHYTOTIC OUTBREAKS OF DISEASE

Chairman : Dr. E. J. Butler (London).

Prof. F. Neufeld (Berlin) : *Bedeutung der Bakterientypen für die Epidemiologie.*

Nach neueren Feststellungen zerfällt eine Reihe früher als einheitlich angesehener Krankheitserreger in Typen, die praktisch konstant sind, d.h. unter natürlichen Verhältnissen nicht in einander übergehen. Die Kenntnis der Typen bildet die Grundlage für das Verständnis der Epidemiologie wie für die Prophylaxe der durch diese Erreger verursachten Krankheiten.

Bei manchen Bakterienarten sind nur wenige, bei anderen zahlreiche Typen gefunden worden ; bei diesen hat man früher zu unrecht angenommen, dass sie besonders variabel seien, ohne scharfe Grenze in ähnliche saprophytische Bakterien übergingen und aus ihnen durch Virulenzsteigerung entständen.

Morphologische, kulturelle, serologische Verschiedenheiten sind ebenso wie charakteristische Unterschiede im pathogenen Verhalten in gleicher Weise zur Unterscheidung von Typen geeignet. Während bei den Tuberkelbazillen die Pathogenität, bei den Pneumokokken die Immunitätsreaktionen für die Bestimmung des Typus entscheidend sind, bieten die Diphtheriebazilleu ein Beispiel für eine Typentrennung auf Grund des kulturellen Verhaltens ; das grosse Interesse, das die Aufstellung der Diphtherietypen gefunden hat, beruht aber darauf, dass diesen kulturellen Unterschieden solche in der krankmachenden Wirkung beim Menschen entsprechen.

Die Aufstellung der Bakterientypen ist aus praktisch-ärztlichen Gesichtspunkten erfolgt ; dieselben Gesichtspunkte sollten auch weiterhin massgebend sein, um zu entscheiden, wie weit wir im Einzelfalle in der Aufspaltung von Krankheitserregern gehen sollen. Die Abgrenzung dessen, was als neuer Typ anzusehen ist, ist zweifellos bisher etwas willkürlich ; es erscheint aber zweckmässiger, zunächst weitere Beobachtungen zu sammeln als voreilige Definitionen aufzustellen.

Wenn auch die Typenforschung aus praktischen Bedürfnissen heraus entstanden ist, so sind doch die tiefen Einblicke, die sie in die chemische Struktur der Mikroorganismen gebracht hat, weit darüber hinaus von grosser allgemeiner Bedeutung, und wir dürfen hoffen, dass auch die Biologen und Botaniker sich allmählich dafür interessieren werden. Ob wir aber jetzt schon auf Grund unserer heutigen Kenntnisse eine Klassifikation unserer Erreger im Sinne der systematischen Botanik vornehmen können, möchte ich

bezweifeln; dazu würde es, wie ich früher schon einmal bemerkt habe, vielleicht doch notwendig sein, zuvor einmal grössere Gruppen der ungezählten frei lebenden Bakterien in ähnlicher Weise zu analysieren, wie wir es jetzt mit einigen Krankheitserregern zu tun im Begriff sind.

Schliesslich sei darauf hingewiesen, dass bereits zwei Entdeckungen von weittragender Bedeutung ihren Ausgang von der Typenforschung genommen haben: die Entdeckung der Kohlehydrate als weit verbreiteter antigener Faktoren durch Avery und Heidelberger und die Entdeckung der willkürlichen Umwandlung eines Pneumokokkentyps in einen anderen durch F. Griffith.

Dr. Homer F. Swift and **Dr. Rebecca C. Lancefield** (New York): *The Problem of Serological Classification of Hæmolytic Streptococci.*

By means of precipitin reactions with suitable sera and the carbohydrate C fraction of hæmolytic streptococci these micro-organisms can be divided into nine or more groups (designated by capital letters) with different properties and habitats. Group A contains most human pathogens; B and C most animal pathogens; the others probably have potential pathogenicity under suitable conditions.

Groups are further divisible into types on basis of immune reactions. Group A types originally rested on mouse protection tests; but subsequently type specific anti-M (protein) precipitins, agglutinins, and opsonins have been shown to run parallel to protective antibodies, so these *in vitro* methods are applicable to type classification (types are designated by arabic numerals). The types in other groups rest on type specific anti-S (carbohydrate) precipitins and agglutinins.

The complex antigenic mosaic of streptococci leads to formation of a variety of antibodies; and while only those against intracellular components are used in serological classification, quantitative variations in these components influence profoundly both the content of the various antibodies in the sera and the amount of the respective antigens in the reagents used in the tests. In Group A streptococci the matt forms (Todd) contain larger amounts of type specific protein M; antibody response to matt forms results in much more potent anti-M precipitating serum than follows immunization with glossy forms. Type specific agglutinins may be induced with either matt or glossy forms and may also be detected with either form; and both kinds of type sera have been used by Griffith in classifying strains from man, with the slide agglutination technique. To compare classifying results of anti-M precipitin and slide agglutination techniques:—

(1) The strains used for preparing M precipitinogen must contain enough M to give suitable reagents;

(2) The sera must contain enough anti-M precipitin to give readable tests.

With these two criteria satisfied the comparative results with the two techniques have run parallel in most instances, but occasional discrepancies have indicated the accuracy of the precipitin tests.

Each technique has obvious advantages and disadvantages: agglutination tests are economic in time and materials, but are liable to give cross reactions, and non-specific results. Anti-M precipitin reactions are more time and material consuming, they are more specific, but detect only the matt or virulent strains.

Following a determination of the group involved, a preliminary survey with slide agglutination technique combined with a final testing of positive agglutinators with anti-M precipitin tests gives the most rapid and certain type specific results.

Dr. Sara E. Branham (Wasbington, D.C.) : *The Significance of Types among Meningococci.*

Various serological classifications of meningococci are summarized and correlated. For practical purposes two broad groups may be recognized : I-III (A) and II-IV (B). These are not sharply defined and innumerable intergradations occur. The members of these groups are not stable, but they tend to change their antigenic patterns during laboratory maintenance. It is therefore of primary importance that standard type strains should be kept true to the classification which they represent if studies made at various times and places are to be comparable with each other.

Charts and tables were shown which indicated the changes in distribution of the various serological types of meningococci during the last 20 years, and the differences in geographic distribution also.

The relation of serological type to tbe clinical manifestations of meningococcic infections, and to prognosis was discussed.

Toxin production seems to be chiefly by some members of the I-III (A) group, and antitoxin is more effective in I-III (A) infections. Definite relations have been noted by various workers between serological grouping and fermentation of carbohydrates, halo formation in agar media containing the homologous serum, and composition of the specific carbohydrates derived from the meningococcus cells. The electrophaetic potentials are correlated with recency of isolation, rather than with serological type. The significance of type in regard to serum therapy cannot be discussed satisfactorily at the present time.

Dr. F. Kauffmann (Copenhagen): *Ueber die Bedeutung der serologischen und kulturellen Methoden für die Typentrennung in der Salmonella-Gruppe.*

Zu einer exakten Typenbestimmung gehören serologische und kulturelle Methoden, die sich gegenseitig ergänzen und die Auf-

teilung der Salmonella-Gruppe in "*Serotypen*" und "*Kulturtypen*" ermöglichen.

Die Serologie bildet das Rückgrat der Typentrennung, da es nur mit ihrer Hilfe möglich ist, ein brauchbares Einteilungsprinzip zu schaffen.

Die Kulturtypen besitzen eine grosse *epidemiologische* Bedeutung, da sie eine weitere Aufteilung der Serotypen in zahlreiche Untertypen ermöglichen. Die *Ammontypen* stellen zwar eine Bereicherung der kulturellen Typendiagnose dar; sie dürfen aber nicht überschätzt werden. Es kommen nicht nur innerhalb eines Serotypus (*S. typhi murium, S. paratyphi C, S. thompson, S. enteritidis*) ammonpositive und ammonnegative Varianten vor, sondern auch innerhalb ein-und desselben Stammes (*S. enteritidis* var. *blegdam*). Die Feststellung der Ammonstärke oder-Schwäche gestattet *keinen Rückschluss* auf die Standortsgebundenheit oder Pathogenität eines Typus, da z.B. unter den hoch menschenpathogenen Typen sowohl ammonstarke (*S. paratyphi B* und *C*) als auch ammonschwache Typen (*S. paratyphi A* und *S. typhi*) vorkommen.

Im Gegensatz zu Hohn und Herrmann betrachte ich die Kulturtypen und speziell die Ammontypen *nicht* als geeignete Basis für die Typeneinteilung, sondern halte die *serologische Diagnose* für *das Primäre*, da die Aufstellung der Kulturtypen erst auf Grund der serologischen Typenbestimmung erfolgen kann.

Prof. G. S. Wilson (London): *The Classification of the Brucella Group on Antigenic Structure and Metabolic Characteristics.*

The use of the absorption of agglutinins test in its original form does not enable a distinction to be drawn between the three chief members of the Brucella group—*Br. melitensis, Br. abortus,* and *Br. suis.* Each organism is able to absorb the agglutinins completely from a serum prepared against either of the other organisms. If, however, a small absorbing dose is used, and is adjusted to the titre of the serum to be absorbed, it is possible to distinguish between *Br. melitensis* on the one hand, and *Br. abortus* and *Br. suis* on the other. Examination of large numbers of strains by this quantitative absorption test has rendered it probable that all three organisms contain qualitatively the same antigens though distributed in different proportions. In the quantitative absorption test the aim is to absorb out the minor agglutinins and leave most of the major agglutinins intact. Such an absorbed serum is practically monospecific and can be used for the rapid differentiation of the two main serological types of Brucella. An exception to this general rule is furnished by certain strains from the South-East of France. Some of these strains react serologically like *Br. abortus*, yet are classified by the dye and H_2S tests as *Br. melitensis*, while others, which react serologically like *Br. melitensis*, have the metabolic characteristics of *Br. abortus*.

In their pathogenicity, the members of the Brucella group exhibit a comparatively high degree of host specificity. Thus the *abortus* type is naturally pathogenic for the cow and the horse, the *melitensis* type for the goat and the sheep, and the *suis* type for the pig. All three types, however, are able to give rise to disease in man, the *melitensis* type being apparently the most pathogenic and the *abortus* type the least. The host specificity is by no means absolute, and a limited degree of cross-infection of animals with unusual types seems to be possible. Loss of the surface smooth antigen is accompanied in all types by a loss of pathogenicity.

All three types of *Brucella* are readily able to infect man; but whereas the majority of infections with *Br. abortus* remain latent, a considerable proportion of *melitensis* infections are accompanied by clinical manifestations of disease. The suggestion is therefore that the *melitensis* type is more pathogenic for man than the *abortus* type, using the term pathogenic not in the sense of being able to cause infection, but in the sense of being able to multiply in the tissues to such an extent as to give rise to clinically manifest disease. The occurence of strains in the South-East of France having the antigenic structure of the *abortus* type, but the metabolic properties and the pathogenicity for man of the *melitensis* type suggests that the greater pathogenicity for man of this type depends on its metabolic characters rather than on its antigenic structure. Additional evidence in support of this conclusion is afforded by a comparison of the American and the Danish *suis* types. These types are antigenically indistinguishable, but differ in their metabolic characteristics. The fact that the American *suis* type is highly pathogenic for man, while the Danish type appears to be comparatively non-pathogenic again suggests that metabolic activity is more important in determining pathogenicity than the exact type of smooth somatic antigen.

Chemical analysis seems to show that both the *melitensis* and the *abortus* types contain the same polysaccharides though in different amount. Substances of protein or polypeptide nature are also present, but unlike the polysaccharides they are highly toxic. There is evidence that the antigenic constituents of the *melitensis* type can be more readily extracted from the organisms than those of the *abortus* type. This fact suggests that the greater pathogenicity of *Br. melitensis* may be associated with a greater solubility in the tissues of its antigenic components. Doubtless the dissolved polysaccharide, though non-toxic, will combine with free antibody and exert a typical aggressive effect, enabling the organisms to multiply more rapidly.

Prof. J. W. McLeod (Leeds): *Significance of Serological and Cultural Types of* Corynebacterium diphtheriæ *in Relation to Epidemic Disease.*

Following lines of cultural differentiation somewhat similar to

those previously suggested by Hammerschmidt (1924) and Parker (1928), the writer, with Anderson, Cooper, Happold and Thompson, differentiated, in the course of 1931 and 1932, three principal and well-defined cultural types of the diphtheria bacillus. For these the names "*Gravis*," "*Intermediate*," and "*Mitis*" were suggested. A full description of these types can be got from the literature.

In the last four years observations on the clinical significance of these types have been made in many towns and in several countries—England, Scotland, Ireland and Germany. The combined figures obtained from a summation of these investigations give the following results:—

Total cases (carriers and rhinitis cases excluded), 5,794.

Gravis, 2,313 cases, 17 per cent. with paralysis and 13 per cent. fatal.

Intermediate, 1,993 cases, 9·9 per cent. with paralysis and 8·6 per cent. fatal.

Mitis, 1,488 cases, 4·5 per cent. with paralysis and 2·3 per cent. fatal.

Since the series is a large one and the cases have been gathered from widely separated areas, it is reasonable to assume that these types, as determined by cultural characters, have clinical significance.

When, however, the combined effects of infectivity and severity of resulting infections are considered, i.e., the diphtheria mortality in the areas in which the different types predominate, the contrast becomes even more striking.

The Gravis form is most definitely associated with epidemic diphtheria, and the Mitis form with sporadic diphtheria.

Type of Diphtheria Infection most numerous in area.	Diphtheria Death-Rate per 100,000 of population.
Mitis	4·5
Intermediate	8·3
Gravis	19·2

There is no antigenic unity within the different cultural types, although a larger number of cases of diphtheria appear to have been associated with one of the immunological varieties of the Gravis strain than with any other.

The chief interest of these observations turns on the question of how far these types are stable and have always been so. In brief, are there three diphtherias due to different bacteria with slightly different but overlapping clinical manifestations, or is there a continuous fluctuation from type to type of which one is more formidable to-day and another to-morrow?

Dr. Helen A. Wright and Dr. May Christison (Edinburgh): *Types of* C. diphtheriæ *occurring in Edinburgh.*

Since 1932 an investigation into types of *C. diphtheriæ* which occur in Edinburgh has been in progress. It has been found that

many strains were encountered which did not conform to the three types *mitis*, " intermediate " and *gravis* described by the Leeds workers, but which could be classified according to the same criteria. The types were designated numerically as follows :—

Type.	Colony Form.	Fermentation of starch.	Virulence.
I	*mitis*	—	+
II	" intermediate "	—	+
III	*gravis*	±	+
IV	,,	—	+
V	,,	±	—
VI	,,	—	—

Prior to April, 1935, 50–60 per cent. of strains isolated from swabs sent in for routine examination were Type II (" intermediate "), 15–20 per cent. were Type I (*mitis*) and Type III (*gravis*) was almost never encountered. 1–4 per cent. were Type IV and the incidence of Type VI rose from 4 per cent. in 1933 to 16 per cent. in the winter of 1934–35. Latterly, however, from October, 1935 to March, 1936, the incidence of both Types II and IV has been approximately 25 per cent. ; 7–8 per cent. of strains have remained unclassifiable throughout.

During the period of the investigation the diphtheria case incidence has been exceedingly low in the Edinburgh area, and the case mortality slight, with a tendency to rise since October, 1935. While, until 1935, Type II strains were isolated from the majority of the severe and fatal cases, latterly 66 per cent. of these have been due to Type IV strains.

The throats of 6,463 healthy school children swabbed during the winters of 1934 and 1935 yielded 1·5 per cent. morphological *C. diphtheriæ*, of which approximately one-third were virulent strains, the great majority of these latter being Type I. Type IV and Type II were seldom isolated from these children. Similarly, swabs sent in for routine examination from suspected carriers rarely yielded Type IV strains and comparatively seldom Type II.

This suggests that a higher degree of immunity in the individual is necessary to protect against infection by these two types than by Type I.

The origin of the various types remains obscure ; but apparently there is a close biological relationship between Type IV and Type III which was prevalent in the severe epidemics reported from various centres in recent years. Type IV is now known to occur in Cape Town and Glasgow, but the epidemiological significance of this type must await the study of further data. On the other

hand the comparatively high fatality rate associated with cases due to Type IV in Edinburgh and its sudden rise in incidence there, suggests the possibility of the onset of an epidemic period in an area where the case incidence of diphtheria has remained low since the beginning of the century.

Prof. K. Poppe (Rostock) : *Diphtheriebakterien bei Tieren.*

In neuerer Zeit sind von verschiedenen Seiten Beobachtungen mitgeteilt worden über das Vorkommen von echten Diphtheriebazillen bei Tieren. Es wurde deshalb die Vermutung geäussert, dass auch Tiere für die Verbreitung der menschlichen Diphtherie in Frage kommen könnten.

Tatsache ist, dass bei Gross- und Kleintieren Bazillen gefunden worden sind, die mit dem Löfflerschen Diphtheriebazillus grosse Ähnlichkeit haben. Da Pseudodiphtheriebazillen (Corynebakterien) bei Tieren nicht selten vorkommen, muss die Forderung gestellt werden, dass nur solche Bakterien als echte Diphtheriebazillen angesprochen werden dürfen, die nicht allein im morphologischen und biologischen Verhalten mit dem Löfflerschen Bazillus übereinstimmen, sondern die auch hinsichtlich der Pathogenität und Virulenz für Meerschweinchen sowie der Toxinbildung sich als Diphtheriebazillen erweisen.

In einigen Fällen ist bei Tieren der Nachweis von echten Diphtheriebazillen, die den Forderungen einer strengen Diphtheriediagnostik entsprechen, gelungen. Soweit die bisherigen Feststellungen ein Urteil zulassen, dürfte damit noch kein Anlass vorliegen zu der Annahme, dass den Tieren eine besondere Rolle bei der Verbreitung der menschlichen Diphtherie zukommt. In Zukunft würde jedoch darauf zu achten sein, ob nicht unter gewissen Umständen eine Übertragung von Tieren auf den Menschen im Bereiche der Möglichkeit liegt.

Prof. W. Campbell and **Dr. E. C. Greenfield** (Cape Town) : *The Incidence of Undulant Fever in Cases of Pyrexia of Uncertain Origin in the Cape Province of the Union of South Africa.*

(1) The Varieties of Brucella isolated by the authors from human patients in the Cape Province have been found to be (*a*) *Brucella abortus*, bovine, (*b*) *Brvcella melitensis*, (*c*) Brucella strains with characters intermediate between these two varieties.

(2) The incidence of undulant fever among pyrexial cases, of a particular class of patients, has been estimated by agglutination tests, the details of which are stated. Agglutination tests have been made of the sera of 661 patients with pyrexia of uncertain origin in whom malaria, typhoid fever, paratyphoid " A " fever, paratyphoid " B " fever, influenza, ordinary pyogenic infections and tuberculosis had been *excluded* as possible diagnoses by clinical and laboratory methods.

(3) Of the patients referred to under (2)—

4·84 per cent. gave serum reactions to a titre of 1 : 400 or more with one or other of three specified strains of Brucella of consistently thermostable suspensibility in physiological saline, employed as antigens of specified density.

9·37 per cent. gave serum reactions under like conditions of test to a titre of 1 : 100 and upwards with one or more of three specified smooth thermostable strains of Brucella.

It is concluded that 4·84 per cent. of such patients certainly and 9·37 per cent. almost certainly, had been or, at the time of investigation, were cases of infection with some variety of Brucella.

(4) Including patients whose sera gave less definite reactions, 15·4 per cent. of the series gave some degree of agglutination. There was a greater frequency of incomplete reactions with sera from patients complaining of arthritic, neuritic and/or muscular pain than with sera from patients without such symptoms.

(5) An effort should be made to standardize the technique of the agglutination test for undulant fever on lines which are acceptable internationally in order to make the results obtained in different areas capable of comparison. In connection with this, it is pointed out that, in spite of certain views to the contrary, no one strain of Brucella is of uniformly consistent value as an antigen capable of detecting agglutinins in *all* cases of undulant fever.

Surgeon Rear-Admiral S. F. Dudley (London): *Bacterial Variation as a Factor in Epidemic Phenomena.*

The many immunity and environmental factors which are also involved make it very difficult to say how far variations in the clinical picture of a disease depend on associated bacterial variations. The average severity of pneumonia is doubtless affected by the serological type of pneumococcus responsible. The extraordinary differences in the incidence of carrier and case infection and of the virulence of meningococcal infections, when all other factors appear constant, can only be accounted for by undetected differences in the characters of the infecting strains of meningococcus. In addition to causing a more severe illness and higher carrier rates than the "mitis" type, the "gravis" diphtheria bacillus can frequently cause carrier infection in Schick susceptible subjects who tolerate it without developing antitoxic immunity, and it would appear loss of induced Schick immunity may occur more frequently in those environments where "gravis" rather than "mitis" is the predominant diphtheria bacillus, *i.e.*, "gravis" is a poor agent for latent "immunization."

Owing to the difficulty of the problem, there is little evidence from the human field that epidemic phenomena are caused by the direct mutation of one bacterial variant into another; but work

on experimental mice epidemics and many laboratory investigations on bacterial variation make it probable that under suitable, even if exceptional, circumstances, bacterial mutation may be an important factor in determining the characters of endemic and epidemic disease.

Dr. Alice C. Evans (Washington D.C.).

The study of hæmolytic streptococci on the basis of sensitivity to phage was undertaken because it offers a new point of view for examining the relationships between the various groups. It now appears that classification on the basis of sensitivity to several races of phage may be useful for routine studies, especially during an epidemic when speed of classification is important. The lytic principle may be preserved indefinitely in a form ready for instant use. Readings may be made after overnight incubation. Classification based on the combined phagological and fermentation reactions is generally well correlated with serological classifications according to agglutinin absorption and precipitin reactions. It is probable that further study will offer explanations for the relatively few discrepancies which occur.

Prof. J. Howard Brown (Baltimore, U.S.A.).

It is a satisfaction to note that in the study of hæmolytic streptococci there seems to be approaching agreement between careful cultural studies and serological grouping. By the study of deep colonies in blood agar plates, fermentation and other biochemical reactions I think it is possible to recognize organisms belonging to Lancefield's groups A, B, C and D in the case of 90 per cent. of the strains studied. On the other hand, the types within the groups are not yet culturally recognizable. It is important that in studies of the streptococci neither the cultural nor the serological aspect shall be neglected.

Dr. H. Loewenthal (London): *The Preparation of Sera and Antigens for the Classification of Hæmolytic Streptococci.*

The classification of hæmolytic streptococci will be greatly facilitated when more is known of the antigenic composition of the cocci. As an example which shows how easily the preparation of type-specific or group-specific sera may then be obtained at will, certain streptococci forming permanent capsules may be mentioned. These cocci—the prototype of which is the well-known Aronson streptococcus—belong to group B1 in Lancefield's classification. A few injections of the capsulated virulent form, either heat- or formalin-killed, yield a highly type-specific serum, whereas the decapsulated and avirulent form, obtained by growing it in type-specific immune serum, produces a group-specific serum.

Again, the preparation of the antigen used in the precipitin test needs improvement. The generally adopted method of extracting the cocci by means of boiling hydrochloric acid yields an antigen apparently somewhat altered in its chemical composition. Thus sera absorbed with such an antigen still retain some activity against the genuine group-specific carbohydrate prepared by alcohol precipitation from bacterial washing fluids. The latter reacts also more readily and in a more specific manner than antigen prepared by hydrochloric acid extraction.

Dr. A. Grumbach (Zürich).

On the communication of Prof. Wilson :

Our Swiss porcine strains also generally form H_2S; rare exceptions are due to dissociation as shown by flocculent growth in liquid medium or mucoid growth on dye plates. Porcine strains seem to be even more infectious and virulent than bovine ones ; our observations show that porcine infections are often more severe and infect 3-5 members of one family, whereas bovine infections are almost regularly sporadic. A hitherto unknown fact is that the porcine strains are easily isolated on agar out of bile blood cultures. That virulence and epidemiological behaviour are more closely related to metabolic than to serological characteristics seems to me to be a general fact as I indicated in my paper on variability.

On the communication of Dr. Campbell :

In order to distinguish actual infections from old ones, we use agglutination and complement fixation test, for it is in our opinion a general rule in Brucella infections that agglutination becomes positive long before the complement fixation test and also disappears first. When we get, therefore, an agglutination of a low degree to, *e.g.*, 1 : 100, accompanied by a strong complement fixation, we consider this the result of an old infection which perhaps was never even manifest, whereas the same agglutinin titre with a negative or slight complement fixation test favours an actual Brucella infection.

Dr. F. Przesmycki (Warsaw).

Our investigations concerning diphtheria carried out with Miss J. Seydel in Warsaw are based on 533 strains. It was found that 234 strains belonged to the gravis type (43·9 per cent.) ; 299 of them belonged to the mitis type (56·1 per cent.). No intermediate strains were found. The gravis strains are more uniform in respect to their biological features. Among the mitis strains a considerable number of atypical strains were traced. The tests for virulence carried out on guinea-pigs revealed that the gravis strains are liable to invade the animal, while the mitis strains are usually found only at the site of injection.

Dr. A. W. Stableforth (London).

Dr. Campbell raised an important point when he referred to a titre of 1 : 400 being taken as indicative of undulant fever in South Africa, using a test Brucella suspension with an opacity equal to that of Brown's tube 3. I have recently reported a comparison of the suspensions, methods of testing and diagnostic criteria used for testing bovines for Brucella infection in seven European countries and in the U.S.A., and have found remarkable differences. In some cases the diagnostic criteria differ widely, but the actual severity is similar; whilst in the case of two others criteria are nominally the same, but the actual severity of one test is five times that of the other. The main cause of these differences is difference in the opacity of the test suspension, but sensitivity of the organisms *per se* and variations in the actual method of setting up the test are also partially responsible. International agreement on methods of testing would be of considerable value.

It would seem from Dr. Campbell's remarks that a similar difference exists in testing for Brucella infection in man, because a titre of 1 : 400 with his suspension (= Brown's tube 3) would give a titre of at least 1 : 800 with the Oxford suspension as used here, and the titre taken as indicative of latent or past Brucella infection in this country is nearer 1 : 80. His test would therefore seem to be one-tenth as severe as that in this country.

Sir Thomas Houston (Belfast).

In 1907 and 1908 Dr. Rankin and myself in Belfast, used a modification of Wright's opsonic technique to diagnose serologically cases of meningococcus infection. This gave reliable results as we got a marked phagocytosis and agglutination within the infected cases while the controls had neither phagocytosis nor agglutination. Epidemic strains from various sources at this time were all Type I; all the epidemic cases of meningitis after they were 3 or 4 days ill gave this reaction, while the Great Ormond Street cases of basal meningitis were different but reacted among themselves (Type II).

The results are fully reported in the *Lancet* and *British Medical Journal* of 1907 and 1908.

Dr. A. B. Wadsworth (Albany, U.S.A.).

Strains of meningococci vary greatly in their biologic characters and in their stability. Some have been under observation more than twenty years and still retain their antigenic and other activities as measured by present standards. Much depends upon how these stock strains are maintained. If the essential conditions of cultivation and animal passage are observed it is not necessary to resort to freshly isolated cultures as has been suggested by a number of observers.

In relation to the classification of diphtheria strains, our studies of the toxigenic activity of these bacilli in synthetic media indicate a marked variation in their inherent capacity for adaptation. My colleague Miss Wheeler has been able, by cultivation in synthetic media, to develop the toxigenic activity of strain 3203, an intermediate form which originally was practically non-toxic, to the point of toxin production equal to that of the Park-Williams strain No. 8. Other strains failed to respond to a similar extent; in fact, there was wide variation—no relation between the vegetative and toxigenic activities—and strain 3203 grew well with, or practically without, toxin production, depending upon the synthetic media selected for its cultivation. Conversely, in the study of attenuation the recovery of toxigenicity in general was increasingly difficult with prolonged attenuation.

My colleagues Miss Sickles and Miss Coffey are now correlating the data that have accumulated from the study of the toxigenic and antigenic activities of the streptococci with the important classifications of Dr. Griffith and Dr. Lancefield. As yet the relationship of the different toxins and their antisera to the groups and types differentiated by the agglutination and precipitation reactions is not clearly defined; but it is important to record the fact that although the toxins exhibit a great variety of types as shown by differences in antitoxic neutralization, it is possible by combination of three types of antitoxin to neutralize all the different toxins of the hæmolytic streptococci of our series.

Prof. H. Zinsser (Boston, U.S.A.) Joint President of the Section, and **Prof. F. Neufeld** (Berlin) then summed up the discussion.

DISCUSSION *on* PATHOGENIC STREPTOCOCCI: RELATION TO SCARLATINA, PUERPERAL FEVER, ERYSIPELAS, TONSILLITIS, ACUTE RHEUMATISM AND INFECTIVE ENDOCARDITIS IN MAN, AND TO MASTITIS, LYMPHANGITIS AND SUPPURATIVE CONDITIONS IN ANIMALS

Chairman: Dr. W. M. Scott (London).

Dr. G. J. Hucker (Geneva, U.S.A.), **Opener.** [Communicated by Prof. R. S. Breed (Geneva, U.S.A.).]

The ubiquitous nature of the streptococci has attracted investigators of varied interests, many of whom have become so isolated and confined to one field of study that little general correlation from the standpoint of the group as a whole has resulted. The wide variety of sources and processes with which the group has been associated has stimulated an undue emphasis by earlier workers

upon habitat and association as a diagnostic character in the identification of the groups and species. Subsequent information and more conservative reflection has revealed that habitat does not serve as a logical and satisfactory basis for fundamental studies. Pathologists have isolated, studied and described species of streptococci from acute and suppurative conditions in man and animals, with little effort to correlate their organisms with strains which no doubt are identical and studied by bacteriologists in other fields. The same criticism also applies to bacteriologists in the field of general and agricultural bacteriology who have studied streptococci from dairy products, fermenting vegetables and fruit products, etc. For this reason there has developed in the literature a large amount of duplication, as well as an unsympathetic attitude between bacteriologists in the various applied fields.

Of fundamental significance is a study of the streptococci as such. In this connection the most important is the relation and differentiation of the various groups. There have been three definite lines of approach to this problem, all of which have contributed to a better understanding of the group. By tradition, bacteriologists have relied upon the more commonly used cultural methods to differentiate organisms. Wide use has been made of these methods, i.e., sugar fermentation and allied reactions, in the classification of this group. More recent investigations have offered two additional lines of approach in distinguishing from a laboratory viewpoint the various species of streptococci, viz., precipitin grouping and ability to be attacked by specific bacteriophages. Both are to be commended inasmuch as they are primarily laboratory procedures and not dependent upon the source or history of the culture and allow the bacteriologist in his own right to identify the species or groups.

Species identification has been further confused by unwarranted minor divisions under the guise of taxonomic studies. Information is now at hand to show that definite broad correlation exists between the cultural, lytic and precipitin results of studies on the streptococci, and if the boundaries of the groups found by such a correlation of these methods of approach are expanded beyond the use of minor and variable diagnostic characters, acceptable and easily recognized groups can be determined. Such groups are well defined. Once such recognizable groups or species are established the public health and pathological significance of the streptococci will be better understood.

Of more fundamental significance than habitat or source, are the reactions which the streptococci produce. Suppurative and acute conditions whether found in man or animals may well be initiated by the same species. The corollary of course may not be true that similar pathologic changes are brought about by the same streptococcus. This is true, for example, in bovine mastitis where more than one type may produce similar symptoms.

Lactic Acid Group.—Only one or possibly two species of true lactic acid streptococci are commonly found in milk, *i.e.*, *Streptococcus lactis* (Lister) Löhnis and *Streptococcus cremoris* Orla-Jensen, which may be very closely related and separated only from the standpoint of degree of flavour production. These types constitute a definite group of streptococci easily recognizable and well defined by broad characters.

Human Fæcal Group.—The most active group of the streptococci and comprising several well-known species and groups all of which should be considered as synonymous, i.e., *Streptococcus fæcalis* Andrewes and Horder, *Streptococcus glycerinaceus* Orla-Jensen and *Enterococcus* Tissier and Martelly. This group is characterized by its rapid reduction of litmus milk prior to curdling, growth at 10° C. as well as at 45° C. and generally failure to ferment raffinose and inulin and a lack of ability to split casein.

Human Mouth Group.—This group may closely approximate to the human fæcal group, but the human mouth group may be differentiated by their failure to reduce litmus milk prior to curdling and ability to ferment raffinose and generally inulin, while practically all strains fail to grow at 10° C. but readily grow at 45° C. Three previously recognized species fall into the group, viz., *Streptococcus salivarius* Andrewes and Horder, *Streptococcus bovis* Orla-Jensen, and *Streptococcus inulinaceus* Orla-Jensen. Included in this group also is *Streptococcus mitis* Andrewes and Horder which heretofore has been considered to represent the common viridans type associated with pathologic conditions.

High Temperature Group.—The characteristics of this group are embodied in *Streptococcus thermophilus* Orla-Jensen. It readily grows at 45° C. and will develop at 50° C., is relatively heat resistant, curdles prior to the reduction of litmus milk, will not grow at 10° C., will ferment sucrose and not attack mannitol, dextrin and glycerol.

The Gelatin Liquefying Group.—Several species have been described which fall into this group, viz., *Streptococcus liquefaciens* Sternberg, *Streptococcus zymogenes* McCullen and Hastings, and *Streptococcus gracilis* (Escherich) Migula. All are no doubt one species. However, if we consider the hæmolysis of blood of importance, then two species, *Streptococcus zymogenes* and *Streptococcus liquefaciens* become significant. The general characteristics of the group are practically those of the human fæcal types to which the gelatin liquefying group is closely related.

The Bovine Mastitis Group.—As a group these organisms do not grow at 10° C. and 45° C., curdle litmus milk prior to reduction, split sodium hippurate and fail to attack esculin. No acid is produced from sorbitol, but generally trehalose is fermented. Three types of these organisms have been described, viz., *Streptococcus uberis* Diernhofer, *Streptococcus dysgalactiæ* Diernhofer, and *Streptococcus agalactiæ* Lehmann and Neumann.

The Pyogenic Group.—The types included in this group are actively beta hæmolytic, fail to split sodium hippurate, ferment trehalose and not sorbitol, do not develop at 10° C. or 45° C., produce a H-ion concentration of *p*H 4·8 to 5·2 in glucose serum broth and from a precipitin standpoint, are known as Group A. Several well-known species should be included in this group: *Streptococcus pyogenes* Rosenbach, *Streptococcus epidemicus* Davis, *Streptococcus scarlatinæ* Klein, etc. All of these types are no doubt identical.

The Animal Beta Hæmolytic Group.—This group is closely related to the human pyogenic types, although it may be separated serologically and become known as Group C. Culturally, they may be differentiated by their ability to produce acid in sorbitol and failure to ferment trehalose.

The Equine Beta Hæmolytic Group—From the precipitin reaction standpoint, this group may be differentiated from the human pyogenic and animal beta hæmolytic group and may be designated as a distinct type in Group C. Culturally, however, it is quickly differentiated by its failure to attack lactose. *Streptococcus equi* holds claim as the designation for the type.

Heat Resistant Beta Hæmolytic Group—This group is represented by one distinct species, viz., *Streptococcus hæmothermophilus* Sherman and Wing. Its chief characteristic is its resistance to heat at 145° F. for 30 minutes. It produces a H-ion concentration in glucose broth from *p*H 4·4 to 4·0, attacks sodium hippurate, ferments maltose, lactose, but not raffinose, inulin or glycerol.

Comments.—In no sense should it be considered that all streptococci fall into the above groups. However, their clear definition indicates that they can be readily recognized by correlating cultural characteristics and constitute sufficiently well-known groups to be considered as adequately defined for practical taxonomic use.

Dr. Perrin H. Long and Dr. Eleanor A. Bliss (Baltimore):
The Significance of Minute Beta Hæmolytic Streptococci.

Minute beta hæmolytic streptococci were first isolated in March, 1933, from the throat of a boy ill with glomerular nephritis. Since then they have been found, at one time or another, in the throats of all patients suffering from acute glomerular nephritis when this disease was initiated by an acute infectious process. They have also been isolated from the rhinopharynges of a majority of patients ill with rheumatic fever and on several occasions they have been found to be the primary ætiological agent of acute and chronic paranasal sinusitis. They have rarely been found in normal individuals or in nephritic subjects the onset of whose disease was insidious.

These organisms are smaller than ordinary beta hæmolytic streptococci and it requires from 24 to 48 hours' incubation before their presence in blood agar plates can be determined. They ferment

the same carbohydrates as do the large beta hæmolytic streptococci. They belong to groups F and G of Lancefield's system of classification of hæmolytic streptococci. Four serological types have been identified. They produce a soluble filterable streptolysin when grown in an appropriate medium but as yet no antistreptolysin for them has been demonstrated.

The sera of patients, in whom these minute streptococci are the primary agents of disease and of those in whom their significance is as yet undetermined (such as nephritic and rheumatic subjects), contain agglutinins for these organisms. In patients ill with acute nephritis, recovery from the morbid process is accompanied by a marked rise in the titre of agglutinins for certain of these minute hæmolytic streptococci.

Dr. Stuart Mudd, Dr. E. J. Czarnetzky, Dr. H. Pettit and Mr. D. Lackman (Philadelphia): *Antigenic Composition and Immunological Behaviour of Hæmolytic Streptococci.*

Phagocytosis of hæmolytic streptococci by washed exudative rabbit leucocytes in the presence of serial dilutions of sera has afforded a sensitive analytical method. The phagocytosis-promoting effects of sera in general have paralleled their mouse protective values. Phagocytosis and protective effects are type-specific for Griffith types, but with some minor cross reactions.

The "C" substance as prepared by Lancefield's method has been found to contain a certain proportion of a lipoid which gives a precipitate with any antiserum prepared against the beta-hæmolytic streptococci; this lipoid cytolyses sheep erythrocytes. Absorptions of Griffith Type I antisera with the fractions of Lancefield's "C" substance, and with "M" substance from a homologous strain fail to reduce their phagocytosis-promoting value; indeed such absorptions increase phagocytosis, possibly due to removal of inhibiting serum lipoids.

A heavy suspension of hæmolytic streptococci may be thoroughly disintegrated by exposure for 1 hour to intense sonic vibration produced by a magneto-striction oscillator of design described by Chambers and Gaines.[1] When the bacterial débris has been thrown down by centrifugalization, the decanted supernatant fluid is found to contain a solute which will completely absorb the phagocytosis-promoting antibody from homologous rabbit antiserum. Acidification with hydrochloric acid to certain characteristic pH values causes precipitation of a fraction which has this property of precipitating the phagocytosis-promoting antibody. The neutralized fluid remaining after such acid precipitation is inactive against the phagocytosis-promoting antibody. The solution resulting from the sonic disintegration may be passed through a Berkefeld filter and preserved by the lyophile process without losing its activity.

[1] *J. Cell. & Comp. Physiol.*, 1932, **1**, 451.

This newly-discovered component is exceedingly labile. It has been deprived of most or all of its antiphagocytic activity by: (*a*) heating to 56° C for 30 minutes; (*b*) keeping in 1 per cent. formaldehyde at neutral reaction for 10 minutes; (*c*) keeping at *p*H 10·0 for 10 minutes. An acid reaction, *p*H 2·0, for 10 minutes only slightly reduced the activity of the labile substance.

Dr. F. Griffith (London): *The Agglutination of Hæmolytic Streptococci.*

The serological classification of hæmolytic streptococci has been helpful in showing the close ætiological relation between scarlet fever and other clinical manifestations of streptococcal infections, as, for example, acute tonsillitis, puerperal fever, etc. The importance in epidemiological studies of a reliable method of identification of a bacterial species is unquestionable, and it will be useful to point out some of the difficulties which have to be overcome in carrying out agglutination tests with hæmolytic streptococci. Sufficient progress has already been made to show that the principal human pathogenic group of hæmolytic streptococci—the *Streptococcus pyogenes* group or Lancefield's Group A—is comprised, like the pneumococcus group, of a number of well-defined serological types. That this was the case was clear from the pioneer work of Dochez, Avery and Lancefield in 1919, and it gave me much pleasure to be able to identify four of their immunological types with types common in scarlet fever and tonsillitis in this country.

The most obvious difficulty in the application of agglutination methods to hæmolytic streptococci is the frequency with which cultures of these organisms form granular suspensions unsuitable even for the rapid slide method of agglutination. Among the various ways of procuring uniform and stable suspensions in fluid media are daily culture passage in plain broth or growth in broth containing 5 per cent. of serum of different species of animals or of ascitic fluid. The disadvantage of serum as well as ascites broth cultures is that some of the streptococcal type-sera do not give with them the rapid agglutination necessary for the slide method. It has been found also that coarsely granular plain broth cultures when centrifuged and resuspended in neat rabbit serum frequently become quite homogeneous and suitable for agglutination tests. The existence of group and type-specific phases similar to the Salmonella phases has been suspected as a cause of the failure to identify serologically certain strains of hæmolytic streptococci. There are no colonial features on blood agar medium which can be relied upon to differentiate between colonies showing group agglutination and colonies showing type-specific agglutination. Type-specific antigen, however, is rarely completely absent from " group " colonies, and the solution of the difficulty of its detection is probably the preparation of high titred type-specific sera.

The production of antisera in rabbits against *Streptococcus pyogenes* offers many problems for solution. Some serological types readily stimulate the formation of good type-specific sera, while with other types the response is mainly to the group antigenic components, and it is difficult to identify in their antiserum any type-specific element. There is little doubt that these differences between types depend on the structure of the type-specific molecule, which in some instances appears to be too readily broken down, either in the preparation of the antigen or after injection into the animal. All the crude type-sera require some treatment to remove group agglutinins, and it is desirable to find some more efficient and less troublesome method than the present absorption with large masses of heterologous cultures. The ideal method would be to use for each type-serum the homologous culture in the "group" phase, but so far it has not been possible to obtain strains devoid of type-specific antigen. Perhaps the most promising suggestion at present is to treat the sera with homologous strains in which the type-specific element has been disrupted by drying and grinding in an agate ball mill.

Dr. C. A. Green (Edinburgh) : *The Serological Types of Hæmolytic Streptococci in Epidemic Scarlatina.*

The bacteriological investigation of strains of hæmolytic streptococci isolated during an epidemic year forms the chief subject-matter of this contribution.

In a series of 1,500 cases, the throat swabs taken on admission in the acute phase of illness yielded 85 per cent. positive cultures, which were serologically grouped into 8 types. Types 1, 2, 3, 4 and 5 are identical with the corresponding numbers of Griffith's series (1935), while three additional types have been provisionally named B, C and D. It has not yet been found possible to place these three types in the above series. The predominant types of this outbreak were 3 and 5, but the monthly percentage of cases due to each of the various types was found to fluctuate markedly. The most striking feature of this variation in type incidence was the gross increase in the number of cases due to types 5 and 7 at the time of maximum extension of the epidemic. Thereafter the epidemic was maintained by a successive increase in other types.

The carrier rate for hæmolytic streptococci in patients on discharge was investigated and found to be 36·8 per cent., but in only 5·4 per cent. was there evidence of persistent heavy infection. Representative strains from such cases were found to be as virulent and as capable of toxin production as strains taken during the early stage of illness.

A study of the age-group incidence of cases revealed no differences in the affected population during an epidemic as compared with non-epidemic years.

Dr. L. Colebrook (London): *Pathogenic Streptococci in Relation to Human Puerperal Infection.*

Three types of streptococci are associated with these infections: (*a*) the hæmolytic group, (*b*) the anaerobic group, (*c*) the non-hæmolytic aerobic group.

The *Anaerobic Streptococci* (strict anaerobes) comprise a large group which cannot be satisfactorily differentiated at present. They present different colonial characters and differ also in respect of gas formation. They are usually of low pathogenicity for animals. Human infections are probably caused by the anaerobic streptococci native to the vagina. They are usually associated with severe injuries to the maternal tissues. Pathological lesions include pelvic cellulitis, thrombophlebitis, lung infarcts, and occasionally endocarditis.

Anaerobic streptococci have been obtained by blood culture (cooked meat and liver digest media) in about 60 cases at Queen Charlotte's Hospital. In more than half the cases the blood cultures yielded more than one type of anaerobic streptococcus, or some other micro-organism in association with the streptococcus (rarely a hæmolytic streptococcus).

Infections by *Hæmolytic Streptococci:* Almost all *severe* infections are caused by Group A (Lancefield) strains. 20–30 serological types have so far been recognized. The predominant 12 types correspond roughly with those most commonly found in infections of the respiratory tract. The infection is essentially invasive in character. It rarely kills, unless it involves the general peritoneal cavity or causes extensive thrombophlebitis.

An important advance in our knowledge of these infections has come recently with the demonstration by Domagk that two diazo-benzene compounds having a sulphonamide grouping (now available under the names of " Prontosil " and " Prontosil Solubile ") have a very definite curative effect on mice infected some hours before by hæmolytic streptococci of high virulence. (Tréfouel, Nitti, Bovet and others in France have since shown that a simpler compound which retains the sulphonamide grouping is equally effective in infected animals.)

The administration of " Prontosil " and " Prontosil Solubile " in human puerperal infections by hæmolytic streptococci has already given remarkable results. Whereas the mortality rate for all cases of such infection at Queen Charlotte's Hospital during the previous five years has varied between 20 and 31 per cent. (average 23·5 per cent.), the rate for the 55 cases treated by these drugs during the past seven months has dropped to 5·5 per cent.

The mode of action of these compounds is still obscure. Although the addition of " Prontosil Solubile " to human blood *in vitro* does not increase its streptococcus-killing power, small additions of amino-benzene-sulphonamide have a very considerable effect. The blood of rabbits treated by sulphonamide—and of

human patients treated by "Prontosil"—shows, however, very little increase of bactericidal power.

Dr. Ronald Hare (London): *Distribution of Hæmolytic Streptococci.*

The streptococci giving areas of β-hæmolysis on blood agar are responsible for a variety of diseases in Man and animals but also occur very frequently as apparently harmless saprophytes. Much evidence shows that all these organisms do not form one species but can be split into nine or more different groups by their morphology, precipitin reactions, and biochemical methods. Lancefield, Edwards, Plummer and Hare have thus shown that the majority of strains from definite infections in Man form one homogeneous group (Group A); that the majority of strains from bovine mastitis fall into a second group (Group B); and that strains causing such infections as adenitis in guinea-pigs, endometritis in mares, and some cases of bovine mastitis fall into a third group (Group C). Strains falling into the remaining groups seldom if ever cause acute infections in Man or animals, although some strains of Group F may be associated with human respiratory infections.

Thus only hæmolytic streptococci with the characteristics of Group A can be regarded as potentially pathogenic for Man. But such strains have a much more limited distribution in Nature than is usually supposed. In a survey, recently carried out, only about one-third of the hæmolytic streptococci found in the human nasopharynx were shown to belong to Group A; strains belonging to this group were not found in normal human fæces; they were absent from the vagina before or after delivery in normal parturient women; they occurred rarely on the skin of the hands, and never on the skin of the back or peri-anal region; although they were present in the dust and air of wards in which infected cases were being nursed, they were not found in the neighbourhood of clinically uninfected persons.

Dr. F. H. Fraser, Dr. H. Plummer and **Dr. D. T. Fraser** (Toronto). [Communicated by Dr. N. E. McKinnon (Toronto)]: *The Erythrogenic Toxin of Hæmolytic Streptococci in Relation to Clinical Source, Serological Classification of Strains and Neutralization by Antitoxin.*

A series of 360 strains of hæmolytic streptococci was examined in an attempt to determine whether any relationship existed between the following factors: source of strain, precipitin group, toxigenicity, neutralization by different antitoxins, and agglutinative type.

Precipitin.—329 strains were of human origin, 31 from other sources. In confirmation of the results of Lancefield and Hare,

the majority of the human strains (89 per cent.) belonged to precipitin group A; of the 270 strains associated with human lesions, 95 per cent.; of the 57 strains from apparently normal throats only 63 per cent. fell into this group. Group A alone contained toxigenic strains; 71 per cent. of the strains in this group produced at least 1,000 S.T.D. per c.c. The strains in Group A were all of human origin except 3 from cows, incriminated in epidemics of sore throat; these 3 belonged by agglutinin absorption to the same serological type as the human strains in the same epidemic.

The strains of human origin, not belonging to Group A, were 17, Group B; 9, Group C; 2, Group F; 6, Group G; 1, Group H.

So far, no overlapping has been noted between agglutinative types in different precipitin groups. 11 strains from precipitin Group C and 66 from Group B were tested with antisera of 9 agglutinative types belonging to precipitin Group A, but the result was negative in every case. Of 123 Group B strains 115 fell into four precipitin types: Ia, Ib, II, III of Lancefield, irrespective of the source of the strains.

Toxin.—Under constant cultural conditions the toxigenicity of a given strain appears to vary little, for strains obtained from different parts of a body, or from various patients in epidemic outbreaks did not show significant differences in the toxins produced. In the Table the toxigenicity of 297 group A-strains is shown in relation to their clinical sources.

Toxigenicity in Relation to Source of Strains

Source of Strains	Number of strains	Toxin <1,000 S.T.D./cc.	Toxin 1,000-	Toxin 10,000-	Toxin 20,000-	Average Toxin S.T.D./cc.
Total Scarlatinal ..	98	19 (20%)	49 (50%)	15 (15%)	15 (15%)	8,300
Total Non-Scarlatinal Infections..	150	35 (23%)	74 (50%)	21 (14%)	20 (13%)	8,300
Apparently Healthy	49	31 (63%)	13 (27%)	2 (4%)	3 (6%)	3,200

Possibly, there is some relation between the type of infection and the strength of the toxin; but, in view of the wide variation of toxigenicity between strains in the same group, little significance can be attached to this. Toxin production did not offer a means of differentiating between strains from different clinical types of disease, e.g., as between scarlet fever and erysipelas. Some strains of each clinical type produced toxin; moreover, among the strains of any one clinical type both weakly and strongly toxigenic strains were found, with the exception of the small number of septic sore throat strains, which were all weakly toxigenic.

Qualitative differences between the toxins of different strains have been demonstrated repeatedly. These differences, on the

whole, are greater between scarlatinal and non-scarlatinal strains, but they also exist within the scarlatinal group.

Serological identification performed with about one-third of the strains showed no apparent association between agglutinative type and strength of toxin produced. Strains of the same agglutinative type yielded widely different strengths of toxin.

Neutralization.—This was tested with 215 strains, in 89 per cent. of which the toxin was neutralized by N.Y. 5 antitoxin, in 8 per cent. by Smith antitoxin, and in 3 per cent. only by a mixture of the two. Neutralization by one or the other antitoxin did not depend upon the strength of the toxin. Toxins neutralized by Smith antitoxin or a mixture of the two were obtained from both scarlatinal and non-scarlatinal sources. Although all the strains tested were neutralizable by two antitoxins singly or combined, they did not fall into two or even three sharply defined groups ; for in addition to those only neutralized by a mixture of the two antitoxins, a few could be neutralized equally well by either antitoxin and still others completely by one antitoxin and partially by the other.

From a practical standpoint, at least in so far as concerns therapeutic application, it is desirable to neutralize as wide a variety of toxins as possible by an antitoxin produced from a minimal number of antigenic strains. This requirement is satisfied by the two strains N.Y. 5 and Smith. Of 145 strains tested both for toxin neutralization and agglutinin absorption, approximately 50 per cent. belonged to 10 agglutinative types. Among the strains neutralized by N.Y. 5 antitoxin, representatives of each of the 10 types occurred ; types B_8 and B_1 were the commonest, comprising respectively $\frac{1}{8}$ and $\frac{1}{7}$ of the strains. Smith antitoxin neutralized only toxins of B_1 strains ; yet some toxins of B_1 strains were not neutralized by it and could be neutralized by N.Y. 5 antitoxin.

Agglutinin Absorption.—Of 237 strains tested 35 per cent. fell into 10 types ; the rest were not further classified. More than half of the strains from scarlet fever cases belonged to 6 types. Types B_1 and B_8 were relatively common in both scarlatinal and non-scarlatinal groups.

Prof. M. H. Dawson and Dr. M. Olmstead (New York) :
Immunological Reactions in Rheumatoid Arthritis.

The work reported results from the observation that sera from the majority of cases of rheumatoid arthritis agglutinate hæmolytic streptococci in high dilutions. The following serological reactions have been studied :

(1) Agglutination reactions ;
(2) Precipitin reactions ;
(3) Antistreptolysin determinations ;
(4) Antifibrinolysin determinations.

(1) *Agglutination reactions :* Sera from over 800 cases of rheumatoid arthritis and an equal number of controls have been examined for agglutinins against hæmolytic streptococci. The results may be summarized as follows :

(*a*) In the majority of cases sera of rheumatoid arthritis patients agglutinate hæmolytic streptococci of Group A (Lancefield) in significantly high dilutions (1 : 160 or higher).

(*b*) With certain exceptions, control sera do not agglutinate hæmolytic streptococci of Group A in significant dilutions. The exceptions concern occasional cases of proven, severe and prolonged infection due to Group A organisms.

(*c*) Rheumatoid arthritis sera do not agglutinate organisms other than Group A hæmolytic streptococci in significantly high dilutions. In particular they do not agglutinate hæmolytic streptococci of Groups B, C, D, E, F and G to a comparable degree.

(2) *Precipitin reactions :* There is a close approximation, but not an absolute agreement, between the capacity of rheumatoid arthritis sera to agglutinate Group A hæmolytic streptococci and to precipitate the group-specific carbohydrate of these organisms. The evidence suggests that these reactions are characteristic for Group A organisms only.

(3) *Antistreptolysin determinations* have been made on over 200 cases of rheumatoid arthritis. The results show that in many early and acute cases this antibody is distinctly elevated. In late and insidious cases the antistreptolysin titre is, as a rule, within normal limits.

(4) The results of *antifibrinolysin determinations* in a large series of cases of rheumatoid arthritis were also reported.

Conclusion : Although these observations do not establish the ætiology of rheumatoid arthritis, they furnish the most incriminating evidence which has yet been brought forward in support of the hypothesis that hæmolytic streptococci of Group A play an important rôle in its pathogenesis.

Prof. K. Diernhofer (Vienna): *Streptokokkentypen bei boviner Mastitis.*

Die von mir in Oesterreich aus 1053 Euterviertein gezüchteten 1057 Streptokokkenstämme lassen sich nach ihrem kulturbiologischen Verhalten gegenüber Lackmusmilch, Methylenblaumilch, Janusgrün, Molybdat, Hippursäure, Aesculin, Salicin, Glycerin, Mannit, Sorbit, Glukose, Laktose, Saccharose, Raffinose, Trehalose, Stärke, Glykogen und Inulin, Haemolyse in Blutbouillon und in und

auf Blutagar *mit* und *ohne* Zuckerzusatz, nach ihrer Pathogenität gegen weisse Mäuse and im Kuheuter in folgende Gruppen einteilen:

(1) *Str. agalactiæ* (1021 Stämme);

(2) *Str. dysgalactiæ* (8 Stämme);

(3) *Str. uberis* (5 Stämme);

Diese drei Gruppen entsprechen den Gruppen I, II und III von Minett, Stableforth und Edwards.

(4) *Str. pyogenes bovis* (14 Stämme) ist die einzige der beim Rinde gefundenen Streptokokkenarten, welche nicht nur im Euterinnern, sondern auch im Gewebe (Subcutis, Interstitium) des Rindes schwere Entzündungsprozesse hervorzurufen vermag. Biologisch ist dieser Gruppe besonders die Vergärung von Stärke und Glykogen eigentümlich, die sie mit einigen Vergleichsstämmen menschlichen Ursprunges (darunter solchen der Gruppen I, II und IV von Griffith) gemein hat, ferner die besonders hochgradige Haemolyse und Mäusepathogenität. Von den humanen Pyogenesstämmen unterscheidet sie sich durch die Fähigkeit, Sorbit, und die Unfähigkeit, Trehalose zu vergären.

(5) *Str. lactis* (3 Stämme);

(6) Sechs "*atypische Stämme,*" sowohl untereinander als auch von allen übrigen Gruppen verschieden.

Morphologisch konnten bei vielen Stämmen glatte und rauhe Parallelformen, bei einigen Agalaktiestämmen drei, nämlich glatte, kleinlockige und grosslockige Formen beobachtet werden. Zwei Agalaktiestämme spalteten farbstoffbildende Formen ab.

Serologisch entsprachen die meisten Agalaktiestämme ungefähr dem Typus *b* nach Stableforth, wenige dem Typus *a* und nur einer dem Typus *c*. Manche Stämme passten zu keinem dieser Typen. Eingehendere Receptorenanalysen ergaben aber bisher mindestens 12 "Agalaktietypen," die eine Anzahl verschiedener, teilweise gemeinsamer Receptoren in verschiedener Kombination enthalten. Die Receptorenanalyse mittels Agglutininabsorption aus einem polyvalenten Serum kann auch praktisch dazu verwendet werden, die Ausbreitung von Galtinfektionen verschiedener Herkunft zu verfolgen, ausserdem zeigte sie bei erfolglos heilbehandelten Tieren mit Streptokokken verschiedener Typen an verschiedenen Eutervierteln an, dass es sich nicht um Neuinfektionen, sondern um Rezidive handelte.

Dr. A. W. Stableforth (London): *The Serological Classification of Streptococcus agalactiæ.*

The streptococci dealt with in this report are the commonest cause of chronic contagious bovine mastitis and are properly called *Str. agalactiæ*. They are similar to those referred to as

" Mastitis streptococci, Group I " in our earlier studies, and include both hæmolytic and non-hæmolytic members, which form a serological group. Certain serological types contain both hæmolytic and non-hæmolytic members. The hæmolytic members all fall into Group B of Lancefield's (1933) group classification of hæmolytic streptococci. The non-hæmolytic members, which form a considerable proportion, react with Group B sera, and would also naturally fall into Lancefield's Group B, if that group classification included non-hæmolytic streptococci. Streptococci of the kind referred to in this report are also, particularly in North America, often referred to as *Str. mastitidis*. It is to be noted, however, that certain streptococci labelled *Str. mastitidis* have not the biochemical characters accepted as those of *Str. agalactiæ*, and also do not react with Group B sera.

In 1932 it was shown by precipitation, slide agglutination, and agglutinin-absorption tests that *Str. agalactiæ* isolated in Great Britain contained three main types—*a*, *b* and *c*. Since 1932, strains from one or more quarters, of 816 cows in 55 herds have been examined. The majority of the strains fell into types *a*, *b* or *c*; two subtypes, a_2 and c_2 were discovered. Over 90 per cent. of these 816 cows were infected with one type only; about 5 per cent. had more than one type; about 3 per cent. had *Str. agalactiæ* of no known type. About 75 per cent. of the cows with a pure infection had β-hæmolytic streptococci only, about 3 per cent. had both β-hæmolytic and non-hæmolytics. A little more than 45 per cent. of the cows were infected with the *a* or a_2 types (nearly all the original *a* type), a little less than 45 per cent. with the *c* or c_2 types (usually the original *c*), and about 10 per cent. with the *b* type.

About half of the 55 herds had one type only, usually the *a* type; whilst in most herds one type predominated. The *b* type occurred in only four herds. Repeated examinations at intervals up to three years showed that change of type in a given quarter is very uncommon. Such change is probably due to a new infection. Where the infecting type is *a* the incidence is usually somewhat lower, but the disease more severe than with *c*. Most strains of *Str. agalactiæ* from samples sent in by practising veterinarians for primary diagnosis, i.e., from severe cases of mastitis, were type *a*. Type *b*, though uncommon, also causes severe disease.

All of about 30 strains of *Str. agalactiæ* from Denmark, Switzerland, Austria and Germany were of type *a* or *b* or closely allied to these types. Diernhofer reports that most Austrian strains are of the *b* type or closely allied to that type.

Comparison of Lancefield's (1934) types of her Group B showed that her types I*a* and I*b* are distinct from our types, whilst her types II and III are allied to our types *a* and *c* respectively but are not identical.

Stewart (personal communication) finds that Australian strains are allied to our *a* and *c* types, but are not identical.

There are thus certain allied types which form broad divisions of *Str. agalactiæ*. These broad divisions are usually readily distinguished and are probably of some epidemiological significance.

When suitable sera are available, slide agglutination, and the group precipitation method, using a ring test, both at room temperature, are quick and reliable methods for the routine identification of *Str. agalactiæ*.

(It was suggested that an agreed nomenclature should be adopted for serological types of *Str. agalactiæ*, and as a result of conversations arising out of the Congress it has been decided that, in order to avoid the possibility of confusion with Lancefield's group classification of hæmolytic streptococci and in conformity with the practice in many other species, the types of *Str agalactiæ* so far distinguished should be redesignated by Arabic numerals, and the relation of the old and new designations dealt with in an article to be published by the author in the near future.)

Sir Thomas Houston (Belfast): *Enterococci.*

The original French authors attribute to the enterococcus potential pathogenicity. Prolonged study of the organism and the variants found on isolation has convinced me that this is correct. The possibility of its serological classification was questioned by Bagger, but Kurt Meyer, by means of Type sera, has divided the strains into types. With Lancefield's technique, Graham showed all enterococci examined to belong to one group.

The Belfast classification employs Type sera obtained with the liquefying forms, which are regarded as the more perfect forms of the coccus. Type I ("*Streptococcus zymogenes*") was shown by Haslett to secrete an S.S.S.; it is a perfect serological type, identical with one of Kurt Meyer's types. Type II secretes a different S.S.S. and also contains group elements. Type III gives a strong group reaction without any S.S.S. With these sera most strains can be classified, though inagglutinable forms occur.

From its natural habitat, the bowel, it can always be isolated; in colitis an active phage is often isolated. The enterococcus is frequently isolated from septic foci, urine, teeth, tonsils, nasal catarrh, sinuses, gall-bladders, abscesses, acne and other skin lesions, and chronic onychias. Agglutinations with the Type forms give evidence of infection. Occasionally the enterococcus has been isolated from the blood and joints. Infected patients are extremely sensitive to enterococcus vaccine.

Much evidence shows that in the bowel and septic foci the enterococcus occurs in a phage-infected form. The best explanation of the variants of the coccus is that they result from phage infection, and the Type forms can only be altered by phage action. Many

so-called viridans strains actually are phage-infected enterococci and can be made to revert to typical enterococci. It is probable that the enterococcus becomes pathogenic owing to its tendency to revert to a more pathogenic form.

Dr. Homer F. Swift (New York).

In antistreptococcal immune rabbit serum the mouse protective power runs roughly parallel with its type-specific anti M precipitin content. Most studies of antibodies in human infections have dealt with non-type-specific antibodies, consequently Hodge and I have investigated the homologous anti-M precipitin curve in the sera of two groups of patients :

(1) Those with uncomplicated streptococcal infections ;
(2) Those with streptococcal infections and rheumatic fever.

We have found that, on the average, the first group developed well marked anti-M precipitins earlier than the second. Patients in Group II with severe streptococcal infections, who early showed strong anti-M titres, seemed to have less severe and shorter rheumatic symptoms than those who developed similar immune reactions later. It, therefore, is possible that recovery is closely associated with effective type specific immunity.

Concerning certain differences in distribution of types in Group B hæmolytic streptococci found by Stableforth and Lancefield, it should be pointed out that most of Stableforth's strains have come from cattle, while many of Lancefield's type 1*a* and 1*b* have been obtained from human sources.

Finally it should be noted that Dr. Mudd's method of obtaining the type specific substance from Group A streptococci is possibly detecting M substance connected with some very labile radical.

Dr. P. Durel (Paris) : *The Clinical Trials of the Sulphonamide Non-azo Derivatives.*

We were very much interested in the remarks made by Dr. Colebrook on the action of chrysoidine sulphonamide. This question, as Dr. Colebrook said, has come very much to the fore recently, following on the work of Fourneau, Tréfouel, Nitti and Bovet, which demonstrated that the activity of the derivatives studied was dependent on the Group SO_2NH_2⬡NR_2 which, moreover, possesses the advantage, from the therapeutic point of view, of giving colourless substances. In Section 5 (p. 297) yesterday, MM. Nitti and Bovet outlined the main points of the research work which led to this conclusion, and we have since reported the results obtained under Professor Lemierre with benzylamino-benzene-sulphonamide. We chose erysipelas for our experiments, as the course of this disease is well known and any modifications more obvious. We

have thus far treated 180 cases of erysipelas. These patients showed no sign of intolerance, and the clinical results, as can be seen from our paper of yesterday, were extremely plain.

Benzylamino-benzene-sulphonamide is insoluble and was given *per os* in the dose of 0·5 to 2 grm. Substances of this series for the present cannot be given by injection in sufficient amount to secure the action which can be obtained by oral administration. We have only reported our trials in erysipelas: clinical experimentation concerned mainly with puerperal sepsis is now going on.

Dr. Perrin H. Long (Baltimore):

Dr. Eleanor Bliss and I have been interested for a year in the question of virulence and type specificity and the immunizing ability of given types of Group A (Lancefield) beta hæmolytic streptococci. We have found that type-specific mouse avirulent types do not produce effective protective or therapeutic antisera in rabbits. A given type must be mouse virulent to produce an efficient antiserum in rabbits. By rapid mouse passage we have been able to raise the virulence of all but two types (Griffith) to a maximum degree. Antisera prepared in rabbits from these types will cure mice of an experimental infection even after a blood-stream infection is present. This leads us to believe that efficient type specific antisera can be prepared. At the present time we are attempting to prepare such sera for use in human infections.

Dr. G. H. A. Buttle (London).

Re Dr. Colebrook's communication: *Mode of action of p-amino-benzene-sulphonamide.* Streptococci will grow from a small inoculum (2 or 3 organisms) in Hedley Wright's medium. p-Aminobenzene-sulphonamide in a concentration of 0·01 per cent. will inhibit the growth of 5,000 organisms for 2 days, and a concentration of 0·1 per cent. will inhibit for a week. Larger inocula are not inhibited. It seems likely that the streptococci are not killed by the sulphonamide; but in blood their growth is held up while the leucocytes destroy them. "Prontosil" does not show this effect, but on reduction an active substance is produced.

Dr. Stuart Mudd (Philadelphia).

The sonic apparatus is purchasable, but expensive.* Dr. Czarnetzky has recently developed another method of obtaining the labile antigen which is less expensive. The streptococci are washed, dried from the frozen state and placed in a mortar. Liquid air is poured directly on them and they are ground with a pestle under liquid air. The necessary precautions against explosion should be taken.

The lability of the antigen in the intact streptococci seems to be less than when in solution. Heating the streptococci at 56° C. for

1 hour sometimes does, and sometimes does not, inactivate the antigen.

The hopes of the pioneers for specific prevention and treatment of disease have been often disappointed. We learned in the first decade of the century that one difficulty was failure to take account of type specificity. Later we learned to take account of variation phenomena. It now appears that the important antigens of typhoid bacilli (Felix), of vaccinia (Craigie), of pneumococci (Goebel, Heidelberger), of Brucella (Huddleston) and of streptococci are exceedingly labile. When we learn to deal with our antigens with less drastic chemical procedures we may find new substantiation for our therapeutic hopes.

Dr. Alice C. Evans (Washington, D.C.).

There are two prevalent erroneous ideas which prevent the recognition of the chronic form of brucellosis. One is that every case of Brucella infection should yield a specific agglutinating serum, and the other is that every case of Brucella infection should show a significant temperature curve. It appears, on the other hand, that in the chronic disease a low agglutinating titre or none, and insignificant rises of temperatures are common.

Convinced that in localities where raw milk from Brucella-infected animals is in use the chronic form of the disease must be a cause of obscure cases of human ill-health, the United States Public Health Service is now conducting surveys in limited areas in several sections of the country. With the imperfect methods of diagnosis available, the results of these surveys cannot be entirely satisfactory, but it is hoped that some valuable information may be obtained. Diagnosis is made on the basis of history and symptoms, and on the phagocytic, agglutinating and intradermal tests.

The most important step to be taken in brucellosis investigations is the development of laboratory methods by which the chronic form of the disease may be more accurately diagnosed.

Dr. Valy Menkin (Boston, U.S.A.): *On the Mechanism of Invasiveness by Pyogenic Bacteria, with Special Reference to the Behaviour of Hæmolytic Streptococci.*

The writer has demonstrated the significance of lymphatic obstruction and the presence of a fibrin network in preventing the spread of various substances, including bacteria, from an acutely inflamed area. The rapidity and intensity with which a micro-organism induces this "walling-off" reaction are an important factor in determining its invasiveness. *Staphylococcus aureus*, owing probably to its highly necrotizing effect on tissues, induces lymphatic blockade already one hour after its cutaneous inoculation, as shown by the inability of trypan-blue to diffuse to the tributary lymphatics from the site of staphylococcus inoculation. With *Pneumococcus* Type I the corresponding time is 6–9 hours. The

fixation of the dye is correlated with the finding of thrombi in lymphatics, and, in the case of *Staphylococcus aureus* and *Streptococcus hæmolyticus*, with the presence of areas containing coagulated plasma. After inoculation of *Streptococcus hæmolyticus* the lymphatic channels remain patent for about 45 hours.

Berkefeld filtrates of *Staphylococcus aureus* induce a " walling-off " reaction in about one hour. The active principle is thermolabile; it resembles leucocidin and is distinct from staphylocoagulase. Filtrates from *Pneumococcus* Type I and hæmolytic streptococci produce no lymphatic blockade.

Staphylococcus aureus localizes readily owing largely to its rapid and intense necrotizing action at its point of inoculation, thus preventing its dissemination. *Streptococcus hæmolyticus*, owing to its mild local effect, freely disseminates to the regional lymphatic nodules and hence infects the organism as a whole. This largely explains the localizing tendency of the *Staphylococcus aureus* in contrast to the invasiveness of *Streptococcus hæmolyticus*. The evidence at hand does not indicate that the fibrinolytic property of streptococci on human blood plays the primary rôle in determining their invasive capacity. Virulence of a micro-organism, as such, remains essentially unaltered by inflammation, and is shown to be a variable independent of invasiveness.

Recent studies have revealed that as the inflammatory reaction progresses in intensity a local acidosis develops and the cytological picture of the exudative material shifts from a preponderance of polymorphonuclear leucocytes to one characterized by a predominance of mononuclear phagocytes. This local acidosis has been shown to be due to a disturbance in carbohydrate metabolism at the site of inflammation. Owing to an increased rate of glycolysis and a depletion of the local alkali reserve, a true lactic acid acidosis develops in the inflamed area. Studies in the same rabbit of lesions induced both by *Staphylococcus aureus* and by hæmolytic streptococci reveal the greater glycolytic activity of *Staphylococcus aureus*. The sugar level in lesions of this micro-organism is found as a rule to be much lower than that in lesions induced by hæmolytic streptococci. This heightened local carbohydrate utilisation serves as chemical evidence, further substantiating the view that *Staphylococcus aureus* owes its greater localizing capacity to the enhanced and more intense inflammatory reaction which it develops at its point of inoculation. In general, the available evidence indicates that the extent of glycolysis in an inflamed area serves as an indication of the intensity or severity of an inflammatory reaction.

Dr. A. Fingerland (Hradec Králové, Czechoslovakia) : *The Occurrence of Streptococci and Diphtheria Bacilli in the Blood of Patients dying of Diphtheria.*

In a fatal case of post-diphtherial myocarditis I observed, in 1931, a marked resemblance of the appearance of the skin veins to that

seen in patients dying of hæmolytic streptococcal septicæmia. The blood of this case, taken shortly before death, contained countless hæmolytic streptococci. This led me to examine systematically the blood of patients who had died of diphtheria. Specimens were taken aseptically from the heart within twelve hours after death. Fifty-six cases were examined from 1932 to 1935. Hæmolytic streptococci were found in 27 (48·2 per cent.). Of 19 patients who had died within a week, 4 (21·1 per cent.), of 37 patients with illness over a week, 23 (62·2 per cent.) gave positive results. In two cases the organism was found in the blood before death, thus proving the streptococcal invasion to have taken place during life. It does not necessarily cause clinical signs, e.g., rise of temperature, but a few cases showed suppurative lymphangitis of the neck.

Diphtheria bacilli were found in the blood *post-mortem* in 3 cases (5·4 per cent.); they were highly virulent for the guinea-pig. In one case the child had died on the seventh day having received antitoxin on the day of its death. In another the child died on the eighth day, having received antitoxin on the fourth day. Another was a case of measles with diphtheritic croup in a child aged seven months who died on the third day.

Diphtheria bacilli, apparently, are present in the blood only exceptionally and in the earlier stages of virulent cases. In the later stages of the disease they were never found, whereas hæmolytic streptococci were present in great numbers.

Dr. L. P. Garrod (London) said that invasion by hæmolytic streptococci had been observed as a terminal event in other infectious fevers, e.g., small-pox.

DISCUSSION *on* MYCOSES IN MAN, ANIMALS AND PLANTS. TAXONOMY. MECHANISM OF PATHOGENIC ACTION. RELATION TO SAPROPHYTIC SPECIES AND CONDITIONS OF SAPROPHYTIC GROWTH

Chairman : Prof. A. E. Boycott (London).

Mr. J. Ramsbottom (London), **Opener.**

In comparing the general symptoms of mycoses in plants and animals and the mechanism of pathogenic action, the essential difference in structure and physiology of the hosts has to be considered.

The ordinary green plant is holophytic and is able to form its own carbohydrates. It does not ingest inorganic food and, apart from osmosis in the roots, all essential processes are carried out through the small stomatal openings which occur principally on the leaves: these (with the lenticels on the bark of trees) are the only parts not covered with a cell-wall. Plants therefore cannot breathe in spores,

and thus there is nothing corresponding to bronchial and intestinal infections. Fungal parasites must enter through the cuticle, through stomata (or lenticels), or *via* wounds. Further, the interior of the plant is composed of cells with definite walls, and there is nothing comparable with a blood stream. It is owing to these differences that the study of animal and plant pathology has travelled along different lines and most markedly in methods of vaccine and serum therapy.

It is unnecessary to discuss the taxonomy of the fungi of plant mycoses. All the pioneer work was done by taxonomists, and the tradition has always been to work in close connexion with systematists. In some groups plant pathology lags behind taxonomy, but in others many of the advances are due to investigations in phytopathology.

On the other hand, the taxonomy of the fungi of medical mycoses is in a bewildering chaos. Many forms have been named in a haphazard manner, with the result that a subject intrinsically difficult has been made repellent to the majority of medical pathologists. However, in recent years the taxonomy of Dermatophytes has been straightened out to some extent, though there is at present an amazing complex of nomenclature. Much work is being done on the asporogenic yeasts and other budding forms often formerly lumped together as "*Monilia*" without any obvious justification. The true yeasts will yield readily to the methods common in laboratories studying industrial fermentations. Characteristic genera such as *Mucor*, *Penicillium* and *Aspergillus* have been the subjects of numerous monographs, and it should be possible to ascertain the diagnostic characters of reputed pathogenic species, though the identification of many of the species described in medical works will remain impossible. Several generic names have been used in an unjustifiable manner, and it remains still to discover what the fungi really are. It is necessary to apply the customary mycological technique and the recognized terminology for any progress to be made.

It is not uncommon for fungi capable of causing plant disease to be able to live saprophytically. Soil-borne organisms are responsible for many root rots, for example, and many of the larger fungi may occur either as saprophytes on dead wood or as wound parasites on the living tree.

The possibility of a saprophytic existence of certain species has not received the same attention in medical mycology, though the fact that Dermatophytes much resemble members of the Gymnoascaceæ has led to the suggestion that these may be coprophilous fungi either originally or still partly. *Ctenomyces serratus*, occurring frequently on the feathers of dead birds, was first recorded on leaves as *Arthroderma Curreyi*; *Gymnoascus setosus* described from an old wasps' nest is *Eidamella spinosa* recorded as causing a ringworm of a dog.

It has recently been suggested that not only can Dermatophytes pass from animal to man and to dead organic matter, but that they are able to cause infection of living plants.

Infections of the feet so often described suggest that the causal fungi can live saprophytically in the soil. Also fungi such as *Hemispora stellata, Oidium albicans, Scopulariopsis Koningi, Aspergillus niger* and many others are typical saprophytes. Indeed, it is possible that most of the fungi causing human disease are facultative parasites.

Dr. E. J. Butler (London): *The Cultural Expression of Morphological Differentiation in Pathogenic and other Fungi.*

The long-continued attempt to find a type medium on which to grow fungi so as to obtain a standardized morphological description for comparative use has failed. In its place has grown up the conception, first applied by Appel and Wollenweber to the *Fusaria,* of the normal and high culture as distinguished from the incompletely developed and young cultures on the one hand, and the degenerate and old cultures on the other. The normal high culture furnishes the best basis for correct classification.

This conception was applied by Sabouraud to the dermatophytes, in which he held that any single medium was scarcely likely to yield that full state of morphological differentiation on which alone a true botanical classification can be based. Langeron and Milochevitch in 1930 and Baudet in 1932 brought out a degree of morphological differentiation hitherto unobtained in many species by growing them on varied natural materials, on agar with polysaccharides, and on agar enriched before autoclaving with cultures of staphylococci.

The fuller expression of morphological characters thus obtained has been effective in reducing greatly the number of genera and species of the dermatophytes, has introduced a welcome simplification into the confused picture of the group, and has vindicated Sabouraud's belief that they are all closely related.

These principles may be equally well exemplified in other groups of pathogenic fungi. Using natural substrata it can be shown, for instance, that *Acladium castellani* Pinoy is morphologically closely allied to *Sporotrichum schencki,* and that the so-called acladiosis should be included in the sporotrichoses.

The pathogenic fungi of warm-blooded animals grow in conditions unfavourable to the full expression of their morphology; to develop this they require, even more, perhaps, than the saprophytic and plant-parasitic species, to be subjected to a cultural technique which will give a normal high culture growth.

Dr. P. H. Gregory (Newton Abbot, Devon): *The Significance of* in situ *Cultures for the Classification of Dermatophytes.*

Over 200 dermatophytes have been described and their classifica-

tion is still in a state of chaos. The hyphal fusion technique of Davidson, Dowding and Buller, in which it was shown that vegetative hyphal fusions could take place between mycelia of similar strains but not between the mycelia of morphologically different species, may prove valuable in testing affinity between different strains of dermatophytes.

Three kinds of criteria have been adopted in classifications of the dermatophytes: (1) spatial relations between spores of parasite and tissues of host (e.g., Sabouraud's genera); (2) macroscopic appearance of colonies on synthetic media (e.g., Sabouraud's species); and (3) spores and other organs which are produced during saprophytic growth in culture, but not while the fungus is parasitizing the skin. Langeron and Milochevitch have emphasized the importance of using as media, substances, such as grains of cereals, which these fungi are likely to encounter in nature. Davidson and Gregory have shown that all the spore forms hitherto known only in culture could be obtained on naturally infected host tissues, scales or hairs, when these were detached from the host and maintained at high humidities at room temperature. No additional nutriment had to be supplied to elicit this saprophytic development, and the cultures were named "*in situ* cultures." It was suggested that the saprophytic spores, formed in moist places on detached infected tissues, serve to disseminate the fungus. The *in situ* culture technique has significance for the classification of the dermatophytes. It emphasizes the importance of the saprophytic spore forms as a normal phase in the life-cycle. By providing a relatively standard "medium" on which the saprophytic phase can be developed, it eliminates the arbitrariness of synthetic media. The most reliable criteria on which a classification can be based are: (1) the relations of fungus spores and host tissue during the parasitic phase; and (2) the spores formed during saprophytic growth on a medium which will reveal the full morphology of the fungus, such as that provided by the *in situ* culture technique.

Miss Dagny Erikson (London): *On the Taxonomy of the Genus* Actinomyces.

The significance of the term *Proactinomyces*, introduced by Haag and adopted by Lehmann and Neumann and by H. L. Jensen, was considered, and the relative merits of the schemes for subdividing the genus, which have been advanced by Ørskov and H. L. Jensen, were discussed.

Prof. W. Brown (London): *Nature of Resistance to Fungus Disease in Plants.*

Many attempts have been made, with varying success, to correlate the resistance or susceptibility of a higher plant to parasitic invasion with the presence or absence of substances toxic or inhibi-

tory to the parasite. While such correlations may in fact be fairly numerous, they are probably only exceptions to the general rule that the resistance of a plant cannot be fully explained on the basis of extracts obtained from it. There is a widely prevailing type of resistance which rests on a capacity of the living tissue to cause, under suitable conditions, a breakdown of the offensive mechanism of the parasite.

A sketch was presented of a line of study which is attempting to explain why certain hosts, which so far as can be seen contain no substance antagonistic to particular fungi and bacteria, are nevertheless able to prevent invasion. This involves a study of the chemical apparatus of invasion, more particularly with reference to the cell-wall-destroying enzyme pectinase. A clear parallelism has been found between the behaviour of organism and enzyme on host tissue, i.e., when the living tissue was presented in a form non-susceptible to invasion by the organism, it was similarly unaffected by the enzyme. In a number of ways it was found possible to modify the living tissue so that it became susceptible to the parasite. When so treated the tissue likewise was attacked by the enzyme.

The effect of nutrient conditions upon the secretion of pectinase enzyme by various fungi and bacteria and the factors which influence the detailed properties of the enzyme of various organisms are being investigated as integral parts of the problem.

Prof. E. A. Pribram (Chicago) : *Biology of Inflammation caused by Yeasts and Fungi.*

The result of the growth of yeasts and fungi in animal tissue is the granuloma. Its origin can be explained by the biology of the invaders. They need : Oxygen, Phosphates, which form 50-60 per cent. of the inorganic ash, Potassium, Calcium, Magnesium, Sodium and Manganese. The organic substances which they utilize are in the first place glucose and proteins. Their parasitic metabolism therefore deprives the human tissue of important nutrient material, thus causing a local deficiency of oxygen and glucose, which are both necessary for tissue respiration. Cells which can adapt themselves to these conditions replace those which, rich in protoplasm, need oxygen. Cells of the reticulo-endothelial system are the only ones which have this ability. By contracting and elongating their protoplasm they form, *gradatim*, histiocytes, fibroblasts, and eventually connective tissue fibres. This contraction of their volume increases their surface, enabling them to absorb even the smallest amounts of oxygen. There is, however, no tendency to form scar tissue, which is the case in secondary proliferative inflammation, because of the presence and the continuous growth and decay of the parasitic cells, which, as we know, can live on their own cells. They not only irritate the surrounding tissue, but they provide it with

sufficient water to prevent dehydration. The "granuloma"—as we call the newly formed tissue, may contain necrotic areas. It is, however, as a whole, a living, proliferating new growth, growing at the expense of the original tissue cells and resembling in this respect the malignant new growth.

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Dr. J. F. D. Shrewsbury (Birmingham) : *The Pathogenic Action of the Common Thrush Fungus in the Rabbit.*

The author's experimental observations on the rabbit with the common thrush fungus, variously known as *Saccharomyces, Oidium,* or *Monilia* (*Candida*) *albicans,* establish the fact that this fungus is lethal for the rabbit under certain conditions. The necessary conditions are inoculation by the intravenous route in doses of a magnitude not less than 60 million living fungus cells per cubic centimetre of inoculum. Different strains of the fungus vary somewhat in their minimum lethal dose, but all are innocuous by any route other than the intravenous. Death is considered by the author to be due to a combination of shock—occasioned by the mycotic embolæmia—and absorption of toxic fungal proteins from the broken-down fungus cells. The kidney is the organ constantly and chiefly affected by the embolæmia, and renal dysfunction is a final sequel if the animal survives the initial embolic shock. Fungal emboli have been demonstrated, however, in most of the organs of the body and also throughout the voluntary musculature. The fungus is incapable of progressive multiplication in, or invasion of, the body tissues of the rabbit, and all attempts to infect that animal by the natural routes were unsuccessful. Sub-lethal doses of the fungus occasion a progressive loss of weight in the inoculated animal, which is not associated with any constant or distinctive alteration in the blood picture, or with any detectable disturbances of function. The fungus cells behave simply as irritant foreign bodies in the tissues and evoke merely a non-specific cellular reaction, composed mainly of small round cells without giant-cell formation, which is restricted to the immediate vicinity of the fungal embolus.

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Prof. E. Pinoy (Algiers) : *Importance des Myxobactéries.*

Comme nous l'avons démontré en 1921 (Ann. Institut Pasteur, t. 35, p. 487) les myxobactéries sont toujours en symbiose avec une bactérie lorsqu'elles donnent leurs fructifications. Elles digèrent la bactérie associée et si l'on ensemence la myxobactérie et la bactérie sur un milieu convenable, on voit tout d'abord se développer la bactérie, puis se montrent des taches négatives absolument semblables à celles produites par les bactériophages. C'est au pourtour ou au centre de ces taches qu'apparaissent les fructifications des myxobactéries.

Nous croyons avoir démontré que les formations rencontrées dans les nodosités des légumineuses, que certains auteurs ont considérées comme des champignons, appartiennent à une myxobactérie. En culture, les myxobactéries donnent des formes en Y exactement semblables à celles que l'on rencontre dans les nodosités.

Une preuve de l'existence d'une myxobactérie dans les nodosités des légumineuses semble être fournie par une expérience d'un de nos collaborateurs, Dr. Lièvre. Il a constaté que si l'on traite des nodosités de fève par un lavage à l'eau oxygénée pour aseptiser la surface, en ayant soin de le faire suivre d'un lavage à l'eau pure stérile, si on les broye ensuite et si on met le produit du broyage sur un papier buvard stérile humidifié contenu dans une boîte de Petri stérile, au bout de peu de temps, il se forme, à l'entour, directement sur le papier, des fructifications d'une myxobactérie (genre myxococcus).

En outre, divers auteurs ont signalé l'existence d'un bactériophage dans les nodosités des légumineuses. Ne viendrait-il pas de la myxobactérie ?

En dehors du rôle certain que jouent les myxobactéries dans les nodosités des légumineuses, on peut se demander si certaines bactéries pathogènes ne constituent pas un stade végétatif de myxobactérie. Par exemple, le staphylocoque donne chez le cheval, plus rarement chez l'homme, une formation spéciale, le kyste du botryocoque, kyste qui ne diffère pas essentiellement de ceux des myxococcus.

Dr. A. Panayotatou (Alexandria) : *Mycoses in Man in Alexandria.*

The author has found in an experience extending over many years that in Egypt fungi may cause affections of the tonsils, tongue, the respiratory and genito-urinary system, as well as the scalp.

1.—*Tonsillomycoses.* In some cases of acute tonsillitis, clinically resembling diphtheria, strains of *Monilia* were isolated in pure culture which gave the biological and chemical reactions of *M. metalondinensis* and *M. tropicalis* Castellani.

2.—*Mycoses of the Tongue.* In one case a penicillium was obtained from the diseased tongue of a child. A similar strain was obtained from the juice of *Ficus nitida*, a very common tree in Alexandria. It was supposed that the child might have acquired the infection by sucking branches of the tree. On Sabouraud's medium the strain grew first as a light down, but subsequently formed a thick, dark greenish to brown wrinkled growth, closely resembling the appearance of the patient's tongue. The fact that *Penicillium* spp. may occasionally become pathogenic is mentioned in Brumpt's " Précis de Parasitologie."

3.—*Respiratory Mycoses.* Such cases, closely resembling phthisis, have been described by the author and other investigators in Alexandria and Cairo. Even the X-ray picture may be suggestive of tuberculosis. A strain of *Monilia* was isolated which resembled *Zeylanica castellani* but differed from it by growing white and giving a negative lead-reaction. It was, therefore, named *Monilia alexandrina.*

4.—*Mycotic urethritis.* The patient had a yellow discharge, simulating gonorrhœa, but *M. alexandrina* was isolated from it.

5.—*Mycotic vaginitis.* From a membranous vaginitis with yellow discharge and thrush-like patches, *Monilia tropicalis* Castellani was isolated. The fungus was pathogenic for guinea-pigs.

6.—*Folliculitis decalvans cryptococcica.* In one such case in an Egyptian woman, yeast-like fungi were isolated belonging to the genus *Cryptococcus* Castellani. The strain was pathogenic for rabbits and white mice; it was agglutinated by the patient's serum in 1:300. Treatment with large doses of potassium iodide and a vaccine prepared from the fungus, which was given subcutaneously and applied to the affected area, proved successful.

Dr. L. P. Garrod (London) disagreed with Dr. Shrewsbury's finding that *Monilia albicans* was incapable of progressive growth in rabbit tissues. He showed a section of a rabbit's kidney after intravenous inoculation in which Weigert staining demonstrated profuse mycelial proliferation of the fungus, and specimens of hilum lesions following intra-tracheal, and massive caseous mediastinal lesions following intra-pleural, inoculations. Dr. Shrewsbury and he were agreed that a number of Castellani's named species were indistinguishable. The human pathologist was admittedly at fault in neglecting the morphological study of fungi, but pathogenicity and serological behaviour were characters on which he necessarily laid greater stress.

Dr. Luis E. Hurtado (Guatemala, Central America): *A Vegetable Culture Medium for Fungi.*

The ripe mango fruit has a very thick layer of pulp which contains sugars dissolved in a very soft paste that can easily be reduced to a dough by grinding. To prepare a solid medium for fungi this dough is added to agar instead of glucose and then sterilized. For subcultures, slices of carefully cleaned raw mango are placed in Petri dishes. The idea of using this material was given by the observation that native boys smear the mango pulp on their face and bodies, and that on each side of their mouth squamous spots of varying size develop. These were found microscopically to contain abundant mycelium. By cultivation on Sabouraud's and mango media penicillia, aspergilli and sterigmatocystes developed. The same species of fungi were obtained by Montoya y Flores in Columbia

(S. America) in colourless squamous efflorescences of the body. The advantages of the mango medium consist in its low price and high vitamin content.

Dr. G. H. Pethybridge (Harpenden, England), with reference to Dr. Gregory's method of *in situ* culture of dermatophytes, said he was struck with the multiplicity of spore forms that arose when the original material was induced to develop further merely by providing additional moisture. He thought great caution would be necessary in the interpretation of the constitution of the resulting growths ; for, unless pure cultures derived from single spores were also raised and studied, there was a danger that serious mistakes might arise. As to Prof. Brown's experiments with the " pectinase " of *Botrytis* on potato tuber flesh, he wondered whether the disintegration of the very thin slice, as compared with the lack of attack on the substantial solid cylinder, might not be due to the fact that in the former, very few layers of living cells would remain, and therefore the natural wound reaction—resulting ultimately in the production of cork cells—might not occur. He thought that the existence of a variety of pectinases would have to be invoked in order to explain the differences in host reaction shown, e.g., by potato tubers when attacked by *Pythium ultimum*, *Phytophthora erythroseptica* and *P. infestans*, resulting in a truly watery, a merely moist, and a quite dry rot respectively.

Prof. E. A. Pribram (Chicago).

" Enzymes " are catalysts causing hydrolysis, or, initiating hydrolysis, i.e., splitting higher molecules into smaller ones by adding water. They must be adsorbed by the cellulose in order to act ; that means, they must be soluble in the cellulose, or, have a higher affinity for cellulose than for an aqueous solution in which they are added. When adsorbed, they lower the surface tension of the cellulose, allowing water to enter.

The equilibrium[1] between: *Enzyme—aqueous medium—cellulose* is also decisive for the differences, mentioned by Prof. Brown, concerning the different resistance of water-imbibed cells and those which contain less water (potato—carrots ; potato soaked with water, potato at higher temperature, at which it imbibes more water than otherwise).

Dr. P. H. Gregory (Newton Abbot).

There is a certain justification for Dr. Pethybridge's criticism as contaminants sometimes appear in the *in situ* cultures. But it has been shown by Spring, by Emmons and by us, working from single spores, that each of the spore types found in dermatophyte cultures on ordinary media do in fact belong to the dermatophyte

[1] Meyer-Overton's conception : "Verteilungsgesetz."

and not to an extraneous organism. Findings based on the new technique must, however, be compared with observations on cultures on ordinary media.

Prof. W. Brown (London) in reply to Dr. Pethybridge, said:

(1) How was the water content of the tissues increased?

By more or less complete injection under the air-pump. The tissue thereby became distinctly more rigid, whence it can be deduced that some of the additional water was taken into the cells. The surplus presumably remained in the inter-cellular spaces.

(2) How thick were the test-discs?

½ mm. as a rule, but 1, 2, 4 mm. were from time to time used.

(3) With these thin discs, were not the cells killed?

No, the discs were rigid and all the cells, apart from those cut, were capable of plasmolysis and, with suitable tissues, could be maintained so for about two days. A feature of the enzymatic attack in some cases was that the cells remained alive and plasmolysable until a very late stage of the enzymatic digestion.

(4) Is the resistance of potato tissue not bound up with cork formation?

It cannot be so in the cases examined, as failure of attack by certain enzymes is settled within a few hours—much too soon for cork formation. Besides, cork formation has been carefully looked for and none found.

(5) How can one explain the differences in the completeness of rotting shown by different parasites?

The differences in detailed properties which have been shown to exist between the enzymes of different fungi and even between enzymatic preparations of the same fungus as obtained under different conditions indicate the answer.

Mr. J. Ramsbottom (London) summed up the Discussion.

DISCUSSION *on* BACTERIA CAUSING ACUTE INFLAMMATION OF THE ALIMENTARY TRACT AND THEIR MECHANISM OF ACTION

Chairmen: Dr. E. J. Butler (London) and **Prof. M. Weinberg** (Paris).

***Prof. E. O. Jordan** (Chicago), *Opener*.

The introduction of certain microbes into the alimentary tract may be followed by injuries conceivably due: (*a*) to the direct penetration of the tissues by living micro-organisms; (*b*) to the effect produced upon the tissue cells by toxic substances formed during the multiplication of micro-organisms in the lumen; (*c*) to the action

of toxic substances liberated through the death and disintegration of the micro-organisms. Injury may also be caused (*d*) by toxic substances produced by certain microbes outside the body and swallowed with the food. In a given case it may be difficult or quite impossible to apportion responsibility between the several factors. This is the case with certain of the attacks of gastro-intestinal disease attributed to Salmonellas.

Injury caused by bacterial products formed outside the body is typically exemplified in botulism where multiplication of the causal organism in the animal body appears to take place only with great difficulty if at all. A second example of poisoning caused by bacterial products has recently come to light in the course of studies on food poisoning carried out in Chicago and other parts of the United States.

It has been found that the sterile filtrate of staphylococcus cultures swallowed by healthy human volunteers in amounts as small as 2 c.c. will cause violent gastrointestinal disturbance, characterized by vomiting, diarrhœa with bloody stools and often extreme prostration. Somewhat larger doses affect rhesus monkeys similarly. This poisonous substance is not a bacterial toxin. It is not destroyed by boiling for 30 minutes, it does not produce a neutralizing antibody when injected into animals and it may cause repeated attacks—20 or more—in susceptible monkeys fed at suitable intervals. The toxic substance is present in the ether extract of a potent filtrate, but not in the residue.

The power to provoke food-poisoning is not limited to any recognizable variety of staphylococcus; strains with various biochemical, hæmolytic and agglutinative qualities have been found capable of generating an enterotoxic substance. Streptococci also have been found to generate a similar substance which possesses, so far as studied, the same characters as the toxic substance in staphylococcus cultures. There is further evidence that some strains of other bacteria, *Proteus*, *Bact. coli*, *Bact. aerogenes*, *Salmonella aertrycke*, produce a similar if not identical substance when grown under suitable conditions.

Old stock strains rarely produce the enterotoxic substance; strains freshly isolated from epidemiologically implicated foods or from pathological lesions are more likely to be toxigenic but are not invariably so. Under laboratory conditions the growth in an atmosphere of about 20 per cent. CO_2 is favourable to the production of the poisonous substance.

It remains to be determined whether potent strains can produce the enterotoxic substance when growing in the alimentary tract. The factors that cause fluctuations in the enterotoxic property of bacteria are still largely to be worked out, as is also the distribution of this property among various groups of bacteria. The nature of the enterotoxic substance and the mode of physiological action are likewise still under investigation.

Prof. O. Lentz (Berlin) [Communicated by Prof. Claus Schilling (Berlin)]: *Die durch Enteritisbakterien hervorgerufenen Lebensmittelvergiftungen und ihre Bekämpfung.*

Der grösste Teil der Krankheitserreger, die Entzündungen des Magen-Darmtraktus verursachen, gehört zur Typhus-Coli-Gruppe. In dieser Gruppe nimmt die Untergruppe der sogenannten Lebensmittelvergifter eine Sonderstellung ein. Die Hauptvertreter dieser Gruppe sind die Bact. enteritidis Gärtner, Breslau, Suipestifer, Voldagsen und Newport neben einer Reihe weiterer seltener beobachteter Typen. Zwar gelangen gelegentlich auch Typhus-, Paratyphus- und Ruhrbazillen und, wie die kleine, aber sehr schwere Berliner Cholera-Epidemie des Jahres 1918 gezeigt hat, auch Choleravibrionen durch Lebensmittel in die Verdauungswege des Menschen und verursachen hier schwere Entzündungen. Aber die so durch Typhusbazillen hervorgerufene Erkrankung wird dadurch nicht zur Lebensmittelvergiftung, sondern bleibt ein Typhus, wie die durch Paratyphus-, Ruhr- und Choleraerreger erzeugte Krankheit stets ein Paratyphus, eine Ruhr, eine Cholera bleibt.

Das Eigentümliche der durch die Lebensmittelvergifter erzeugten Erkrankung ist, dass nicht die *Infektion* des Erkrankten, die zwar nebenher auch vorhanden sein *kann*, das Krankheitsbild beherrscht, sondern vielmehr die *Intoxikation* durch die schon in dem infizierten Lebensmittel gebildeten spezifischen Gifte.

Die allgemeine Auffassung geht daher dahin, dass diese Bakterien für den Menschen im allgemeinen nicht pathogen sind, sondern für ihn nur durch die in infizierten Lebensmitteln gebildeten Gifte gefährlich werden.

Dagegen besitzen die Lebensmittelvergifter eine ausgesprochene Pathogenität für gewisse Tiere. So sind viele von ihnen als Erreger typhus-und ruhrartiger Erkrankungen sowie des infektiösen Abortus unserer Schlachttiere, Rinder und Schweine, der Kolik der Pferde, infektiöser Darmerkrankungen unseres Hausgeflügels und freilebender Schwimmvögel sowie von typhusartigen Erkrankungen der Nager, vornehmlich der Ratten und Mäuse, bekannt. Die bakteriämische Natur dieser Enteritiden bedingt es, dass das Fleisch so erkrankter Tiere die Krankheitserreger enthält. Wenn nun ein erkranktes Rind, Schwein, Pferd oder Geflügel notgeschlachtet wird, so können sich während des Abhängens des Fleisches die in ihm enthaltenen Bakterien vermehren und ihre stark wirkenden Toxine bilden.

Aber auch in die Milch enteritiskranker Kühe und in die Eier enteritiskranken Geflügels gehen die Krankheitskeime über. So können auch durch sie Vergiftungen hervorgerufen werden, wenn z.B. Milch zu Schlagsahne oder Käse verarbeitet, Eier roh oder nach nur mässiger Erhitzung genossen oder Milch wie auch Eier zu anderen Speisen, Speiseeis, Creme oder Backwaren hinzugefügt werden.

Auch können von infizierten Ratten und Mäusen die Keime auf an sich einwandfreie Lebensmittel übertragen werden.

Eine starke Verbreitung haben die Lebensmittelvergifter dadurch erfahren, dass sie als Ratten- und Mäuseschädlinge vielfache Verwendung zur Vertilgung dieser schädlichen Nager gefunden haben. Zahlreiche Epidemien von Lebensmittelvergiftungen bei Menschen sind in den letzten Jahren beobachtet worden, die ihre Entstehung der Auslegung bakterieller Ratten- und Mäusevertilgungsmittel und dadurch herbeigeführter Infektion von Schlachtvieh, Rindern und Schweinen, und Geflügel, vor allem Enten, verdankten. Ich habe mehrfach in den Jahresberichten der preussischen Medizinalverwaltung über derartige Epidemien berichten können.

Da bei den bakteriellen Lebensmittelvergiftungen die das Gift erzeugenden Bakterien letzten Endes stets von einem kranken Tier stammen, muss die Bekämpfung dieser Krankheiten stets auch die entsprechenden Erkrankungen der Tiere in Betracht ziehen. An diesem Kampfe sind also Humanmediziner wie Veterinäre in gleicher Weise interessiert. Deshalb bestimmen auch die Ausführungsbestimmungen zum preussischen Seuchengesetz, dass bei der Bekämpfung der bakteriellen Lebensmittelvergiftungen der beamtete Arzt mit dem beamteten Veterinär Hand in Hand arbeiten soll. Nur durch die energische Bekämpfung dieser Erkrankungen beim Schlachtvieh und Geflügel kann den Lebensmittelvergiftungen bei Menschen wirksam vorgebeugt werden.

Auch müsste die Verwendung bakterieller Ratten- und Mäusevertilgungsmittel auf gesetzlichem Wege unterbunden werden, zumal die Wirksamkeit dieser Mittel unzuverlässig ist und zahlreiche sicherer wirkende chemische Vertilgungsmittel zur Verfügung stehen.

Wenn auch der Kontakt von Mensch zu Mensch bei der Verbreitung der Lebensmittelvergiftungen nur eine untergeordnete Rolle spielt, so behalten die in der Seuchenbekämpfung bewährten Massnahmen, die Absonderung des Kranken und die Desinfektion seiner Abgänge, im Interesse der besseren Pflege des Kranken und zur Verhütung einer Uebertragung der in seinen Exkrementen enthaltenen Krankheitserreger auf empfängliche Tiere ihren Wert.

Von besonderer Wichtigkeit ist aber die schnelle exakte bakteriologische Feststellung der bakteriellen Lebensmittelvergiftungen. Dank der Feststellungen der Kieler Schule sowie der Untersuchungen von Boecker und Kauffmann macht die Differenzierung der Lebensmittelvergifter heute keine Schwierigkeiten mehr. Deshalb muss, unbeschadet der vom wissenschaftlichen Standpunkt nicht zu bestreitenden nahen Verwandtschaft der Lebensmittelvergifter mit den Paratyphusbazillen, aus Rücksicht auf die praktischen Bedürfnisse gefordert werden, dass die Lebensmittelvergifter durch die Bezeichnung als Enteritisbazillen als eine besondere Untergruppe der Typhus-Coli-Gruppe gekennzeichnet werden. Nur durch die regelmässige Verwendung dieser Nomenklatur kann der bakteriologische Diagnostiker den Praktiker (beamteten und praktischen Arzt und Tierarzt) vor Missverständ-

nissen und vor diagnostischen, therapeutischen und epidemiologischen Irrtümern bewahren und die strenge Unterscheidung der toxischen Lebensmittelvergiftungen von den infektiösen Paratyphuserkrankungen des Menschen zum Gemeingut der Aerzte machen.

Major J. S. K. Boyd, R.A.M.C. (London): *New Types of Dysentery Bacilli.*

Experience in India shows that there are many organisms having the morphological and cultural characters of dysentery bacilli which differ in their antigenic pattern from the four accepted types, *viz.*, Shiga's, Schmitz's, and Sonne's bacilli, and the Flexner group as defined by Andrewes. The majority of these atypical organisms are related to the Flexner group.

Collections of strains have been made and investigated, and nine new types, or races, of dysentery bacilli allied to the Flexner group have been identified. Of these, two resemble Andrewes' types closely: one of them (103) probably, corresponds to one of the original Y strains. A third (88) is identical with the "Newcastle" bacillus in its antigenic pattern, but differs in its biochemical reactions. The remaining six types, of which one is of common occurrence, are of distinct and separate antigenic pattern.

In the military laboratories of India, from 1932 to 1935 inclusive, 7,339 strains of dysentery bacilli were isolated. 1,121 strains (15·3 per cent.) were of these new types. Only 49 strains could not be identified.

In the following analytical table the "new" types are designated by the laboratory index number of the type strain.

ANALYSIS OF 7,339 STRAINS OF DYSENTERY BACILLI ISOLATED 1932-35.

Non-Mannite Fermenters.		Mannite Fermenters.															
		Late Fermenters of Lactose and Saccharose	Non-Fermenters of Lactose and Saccharose														
					Other Types												
Shiga	Schmitz	Sonne	Inagglutinable with Sonne Serum	V. W. X. Y. Z, etc. (Andrewes' Series)	103	P.119	170	88 *	P.288	P.274	D.1	D.19	P.143	Total of these other Types	Inagglutinable with any of these Sera	Not Investigated
No. 1,050	No. 402	No. 801	No. 135	No. 3,686	No. 199	No. 131	No. 190	No. 371	No. 64	No. 75	No. 67	No. 8	No. 16	No. 1,121	No. 49	No. 95
% 14·3	% 5·5	% 10·9	% 1·8	% 50·2	% 2·7	% 1·8	% 2·6	% 5·0	% 0·9	% 1·0	% 0·9	% 0·1	% 0·2	% 15·3	% 0·7	% 1·3

The Y type mentioned above (Type 103) produces in artificial culture a variant which is serologically identical with the strain of Y at present used in Army laboratories; each completely absorbs the serum of the other. Further, this variant absorbs all heterologus agglutinins from serums for W, Z, and those of the "new" types which cross-agglutinate with Andrewes' V-Z series. In the case of V serum, after absorption a residue of Z agglutinin remains in addition to homologous V agglutinin. The result, in the case of serum prepared from the existing type strain of X, is anomalous.

It is considered that this variant represents a group or non-specific phase, and that the Flexner dysentery bacilli in most cases contain each its own specific antigen plus a varying proportion of this non-specific antigen; and not, as postulated by Andrewes, a mosaic of specific antigens.

Classification of members of the group, which should be according to specific antigen, is greatly simplified by the application of this finding.

Dr. Marguerite Altoff (Paris) : *Bacille dysentérique, agent pathogène probable d'une entérite epidémique de nouveaux-nés.*

Nous avons isolé des selles de nouveau-nés atteints d'entérite grave un bacille dysentérique, présentant les caractères morphologiques et biochimiques de bacille de Flexner, tandis que sa virulence pour les animaux de laboratoire le rapproche du bacille de Shiga. Nous avons pu isoler par filtration de vieilles cultures en milieu liquide: (1) une *exotoxine*, capable de produire chez le lapin une réaction violente, mais passagère, et (2) une *endotoxine*, lysat de cultures jeunes dans l'eau distillée, qui reproduit chez l'animal les symptômes de l'infection et aussi les lésions anatomo-pathologiques.

Nous avons obtenu constamment avec l'endotoxine une réaction de Shwartzman positive. Celle-ci était négative lorsque l'injection préparante et déchaînante étaient faites avec l'exotoxine. Mais elle était positive lorsque l'une des deux injections était faite avec l'endotoxine.

Nous avons fait ingérer aux lapins à jeun de l'endotoxine, ce qui était très bien supporté. Mais une injection intraveineuse d'un quart de dose mortelle de cette même endotoxine, pratiquée 24 heures après, provoquait la mort de l'animal avec des phénomènes de dyspnée intense et des convulsions. L'animal semblerait être sensibilisé par l'ingestion préalable d'endotoxine. A l'autopsie nous trouvions des foyers hémorragiques dans les reins et dans les poumons; très souvent aussi des hémorragies péritonéales. L'endotoxine ingérée aurait traversé la paroi intestinale pour se fixer sur différents organes en produisant une sorte de mordançage préparatoire pour l'injection intraveineuse qui va suivre et qui, elle, déclanchera la réaction hémorragique. C'est un phénomène de Shwartzman, dans lequel la sensibilisation locale

primitive se ferait non pas par injection intracutanée, mais par simple ingestion.

Prof. M. Weinberg (Paris): *Vaccination anticolibacillaire; les holovaccins.*

J'adresse mes félicitations à Madame Aïtoff pour la très intéressante communication qu'elle vient de nous faire.

Je me permettrai, à cette occasion, de vous exposer en quelques mots comment les recherches poursuivies ces derniers temps ont modifié nos idées sur la préparation et l'efficacité des vaccins antimicrobiens. Ces vaccins ont donné, en général, de bons résultats; il suffit de rappeler le succès de la vaccination antityphique par le vaccin T A B. Cependant, l'emploi de ces vaccins a réservé quelquefois de cruelles déceptions.

Cela tient à ce qu'il n'a pas été suffisamment tenu compte du fait, mis surtout en évidence au cours de ces dernières années, que certains microbes sécrètent des toxines multiples. D'autre part, il ne faut pas oublier que certains microbes, tels que le colibacille, le b. dysentérique, les bacilles typhique et paratyphique, etc., agissent plus par leur endotoxine que par leur exotoxine.

Je soutiens, depuis la guerre, qu'un bon sérum thérapeutique doit être à la fois antimicrobien et antitoxique, autrement dit anti-exo-endotoxique.

Il en est de même pour les vaccins. Ceux-ci doivent être préparés en tenant compte de tous les antigènes toxiques produits par un microbe donné. Ils doivent être à la fois anti-exo-endotoxiques (holovaccins, d'après notre terminologie).

Prenons un exemple. Vous savez qu'on obtient difficilement chez l'homme une immunité anticolibacillaire au moyen de vaccins préparés avec les corps microbiens, chauffés ou formolés.

Nous avons pratiqué, en collaboration avec Mlle Kreguer, un grand nombre de recherches sur la vaccination des animaux avec des antigènes colibacillaires très différents: corps microbiens chauffés, formolés, anaculture, endotoxine seule ou mélangés avec l'exotoxine.

Voici en deux mots, nos résultats: la vaccination la meilleure, l'immunité la plus solide, sont obtenues soit par l'anaculture, soit par l'endotoxine.

Je dois ajouter que nos essais chez l'homme de vaccination anticolibacillaire ont été nombreux et qu'ici encore ce sont l'anaculture et l'endotoxine qui nous ont donné les meilleurs résultats.

Dr. R. Lovell (London): *Colibacillosis of Calves.*

Bact. coli is responsible for a large proportion of deaths among calves during their first week or so of life, the typical syndrome being known as "white scours." Of 100 calves collected at random and examined shortly after death, *Bact. coli* was responsible for

death in 37 and involved in six further cases. All strains examined were indole +, M.R. +, V.P. —, Eijkman +, citrate —, and failed to liquefy gelatin.

Certain strains showed a rapid mutation when grown on agar for several days, a grey-translucent growth appearing at the periphery of the parent opaque mucoid colony. Mucoid colonies consist largely of capsulated bacteria, which are not agglutinated in buffer mixtures of lactic acid and sodium lactate (*p*H 4·8 to 2·4) and are agglutinated to low titre by homologous serum. Grey-translucent mutants consist largely of non-capsulated bacteria, which are agglutinated in buffer mixtures of lactic acid and sodium lactate and agglutinated to high titre by homologous and some heterologous sera also. By using mucoid strains both for producing immune sera and for agglutinating suspensions, specific reactions are produced.

A carbohydrate extract, soluble in water, giving positive Molisch and negative Millon reaction, can be prepared from both variants by preliminary alkali extraction at 70° C.; that prepared from mucoid strains is specific by the precipitin test, whilst that from grey-mutants is apparently less specific and less in quantity. The precipitin method, using extracts and sera prepared by injection of whole cultures, is being applied in an attempt to identify those strains of *Bact. coli* which are capable of producing disease in calves. It is also applicable for strains which do not show the rapid mutation. The main object is to determine whether special races of *Bact. coli* produce white scours in calves and, if so, to what extent.

Eight different sera have been used and it has been found found possible by precipitin reactions to group 78 of 110 strains; actually 22 strains fell in one group, 22 in a second and 34 in the remaining six groups.

It seems likely, though the numbers of observations are still small, that (1) special races of *Bact. coli* capable of causing disease and death in young stock do exist, (2) that in some instances more than one such race may exist in a particular herd and (3) that usually one type only is concerned in the death of an individual calf.

Dr. A. D. Gardner (Oxford): *Cholera and its Bacterial Cause.*

The three chief conditions for microbial infection of tissues are:

(1) Suitable food and environment for the microbe. For *Vib. choleræ* the alkalinity of small intestine and bile are especially favourable.

(2) Self-protection of the microbe. A protective external layer is characteristic of the smooth, virulent phase.

(3) Toxic action on living cells. In *Vib. choleræ* the endotoxin repels and destroys phagocytes, and by its action on the splanchnic nerves disorders the movements of the intestine.

The route of entry is probably through the stomach. Sanarelli's route, *via* pharyngeal lymphatic system, blood-stream and intestine, may also operate at times. The last stage of this may be through the bile (Greig). Sanarelli's contention that normal inhabitants of the intestine, e.g., *B. coli* or *Proteus*, play a part is unproven ; the Sanarelli phenomenon is probably similar to Shwartzman's phenomenon, and there is no direct proof that it plays a part in cholera. The fact that it may be elicited with El Tor vibrios (Doorenbos) does not prove these to be choleriginic in man.

The El Tors differ from *Vib. choleræ* in producing an exohæmolysin for sheep or goat erythrocytes, a property which the speaker believes to be fixed and specific. The hæmolysis by phage-resistant cholera cultures, observed by Doorenbos, is not easy to produce ; the writers initial failures suggest that it is of a different order of phenomena to El Tor-hæmolysis, and is not a formidable obstacle to identification. Chemical-analytical differentiation (Linton *et al.*) may prove feasible, though as yet the proof of different proteins is unconvincing owing to failure to distinguish between known serological entities among strains hitherto called El Tor. Serological classification by O-agglutination (Gardner and Venkatraman) is at present indispensable.

Dr. A. Panayotatou (Alexandria) : *Colibacillary Infections in Man in Alexandria.*

B. coli, although a normal inhabitant of the intestine from the first hours of life, may under certain circumstances become one of the most dangerous pathogenic microbes. The following cases of "Colibacillosis," observed in Alexandria, might well have been confused with other microbial infections.

Case 1.—Four days after a dietary indiscretion, high fever, vomiting, diarrhœa and intense headache set in. Tongue dirty, fetid liquid stools, vomit bile coloured, temperature 40·3° C., pulse 120. Strongly hæmolytic *B. coli* were isolated from the blood as well as the stools and urine. All the strains were agglutinated by the patient's serum up to 1 : 500. Treatment with a stock vaccine of *B. coli*, *per os*, was successful in six days.

Case 2.—Temperatures between 38 and 39° C., headache, mild diarrhœa alternating with constipation. Widal negative for typhoid and paratyphoid bacilli, diazo-reaction negative, urine contained *B. coli*. Treatment with coli vaccine *per os* was successful in four days.

Case 3.—Chronic diarrhœa, following a dietary indiscretion. The condition had persisted for 20 months. Bacteriological and parasitological examination was negative. The patient's serum agglutinated his own *B. coli* up to 1 : 600. Treatment with Vincent's serum was successful within a few days and the patient remained well for three months.

Case 4.—Woman of 23 with acute pain in the left hypogastrium, simulating appendicitis. The urine contained *B.coli.* Treatment with coli vaccine *per os* was successful within a few days and the patient remained well for three months.

These cases prove that infections with *B. coli* can imitate various infectious diseases.

Prof. M. Weinberg (Paris) : *Sérothérapie par voie buccale.*

L'observation très intéressante que vient de nous rapporter Mme Panayotatou m'incite à vous résumer, en quelques mots, les recherches que je poursuis depuis de longues années sur la sérothérapie par voie buccale.

Déjà à la fin de la guerre, j'ai eu l'occasion de guérir quelques cas d'infection putride de l'intestin par l'ingestion de sérum anti-gangréneux polyvalent, qui renferme, entre autres, des anticorps anti-perfringens et anti-sporogenes. Actuellement, je fais absorber aux malades un mélange de sérums antigangréneux polyvalent, anticolibacillaire et de sérum complémentaire (préparé contre les microbes secondaires, mais qui jouent souvent un rôle important dans l'évolution des appendicites graves et, en général, des infections intestinales (*B. fusiforme, Staphylococcus parvulus, B. funduliformis,* streptocoques anaérobies et aérobies, etc.).

Il arrive quelquefois, qu'il est impossible d'opérer un sujet atteint d'une appendicite grave ou d'injecter du sérum sous la peau, par crainte de phénomènes anaphylactiques. Dans ces conditions, il m'est arrivé de soulager et même de guérir les malades, en leur faisant ingérer plusieurs jours de suite le mélange de sérums indiqués plus haut.

Je fais absorber du sérum à jeun (50 c.c. du mélange : sérum antigangréneux—20 c.c. ; sérum anticolibacillaire—20 c.c. et sérum complémentaire—10 c.c.) au moins deux heures avant le premier repas du matin. Quelquefois, le malade accuse une amélioration incontestable le jour même de l'ingestion. Dans d'autres cas, on est obligé de faire prendre au malade du sérum plusieurs jours de suite.

Il est bien entendu qu'en principe, on ne doit pas substituer l'administration du sérum par voie buccale aux injections sous-cutanées ou intraveineuses, mais le praticien doit savoir que l'ingestion de sérum peut rendre de grands services dans les cas exceptionnels où l'état du malade ne permet pas une intervention chirurgicale et où il y a des craintes justifiées d'accidents anaphylactiques qui sont infiniment plus rares à la suite de l'introduction de ce produit thérapeutique *per os.*

Dr. P. Kouchakoff (Lausanne) : *The Intestinal Flora and its Rôle in the Life of Man and Higher Animals.*

The rôle and significance of the intestinal flora are as yet insufficiently studied : in fact, it is not known whether it is necessary

at all. During his sojourn in the Polar regions—in Northern Siberia, Novaya Zemlya and in Franz Josef land—the author has devoted eight years to the study of this question, having examined more than 100 white bears and 1,000 birds. In the bears only *B. coli commune* was discovered, while in sea-gulls and migratory birds there was a considerable and varied intestinal flora. On the other hand, in the local polar birds the flora became progressively poorer as one approached the North Pole. Thus, in *Uria* sp. (Auks) the intestine was found to be perfectly sterile (more than 300 observations).

Experiments with bear-cubs a fortnight old, kept together with dogs, showed that the intestine of the bears was not immune against the group of Gram-positive microbes, and that invasion by enterococci and other microbes always caused in these animals marked intestinal disorder, sometimes with fatal results (3 out of 8 died).

Uninterrupted observations in the course of two years, demonstrated that in bear-cubs fed on a human diet and having an abundant intestinal flora, growth and general development were retarded, as compared with wild cubs of the same age, in spite of the fact that they lived in complete freedom, under identical polar conditions.

It is to be concluded that the organs of digestion of the higher animals are capable of functioning properly in the absence of intestinal microbes provided the organism receives adequate nutrition, in the form of natural foodstuffs.

The consumption of food altered by high temperatures and by complicated manufacture causes a disturbance of the functions of the digestive organs, and brings about the intervention of the microbes. The biological processes of these bring about the destruction and assimilation of the food-residues which failed to be assimilated physiologically.

The presence of an abundant intestinal flora is an undesirable phenomenon, since the formation of by-products of metabolism produces a chronic intoxication of the cells of the organism consequently leading to a diminution of its resistance both to the intestinal and other microbes. In other words, microbes which are saprophytic in the healthy organism become pathogenic in the weakened organism.

Prof. H. Braun (Istanbul).

Eine internationale Einigung über die Einteilung der Dysenterie-Bazillentypen ist erwünscht. Nach meiner Meinung wären nach dem heutigen Stande vier Typen zu unterscheiden: I. Shiga-Kruse, II. Flexner, III. Kruse-Sonne und IV. Schmitz. Zur Klassifikation müssen herangezogen werden: Morphologie, Ernährungsphysiologie, Antigene und Pathogenität. Sehr geachtet muss auf saprophytische Stuhlbakterien werden, die Dysenteriebazillen ähnlich sind und sie daher vortäuschen können. Prüfung der Beweglichkeit bei Zimmertemperatur gezüchteter Kulturen ist erforderlich. Diese sind bei

Saprophyten öfters beweglich, während 37 Grad C.-Kulturen unbeweglich sind. Einführung von Standard-Nährböden für die Prüfung der ernährungsphysiologischen Eigenschaften der Bakterien ist wünschenswert.

Prof. W. Campbell (Cape Town).

Final Classification of Dysentery Bacillus Types.

This must be based on a summation of characters comprising (*a*) cultural features on standardized media, (*b*) metabolic and biochemical functions, (*c*) antigenic constitution, as stated by Braun, and not on (*a*), (*b*) or (*c*) alone. The capacity of a type to produce exotoxin will provide a demarcating line between the paradysentery organisms of greater importance than has been recognized hitherto; Altoff's toxigenic "bac. dysentérique des nouveaux-nés" illustrates this.

Salmonella Organisms in Food Poisoning and Dysentery.

The antigenic similarity of certain Salmonella strains to some paradysentery organisms, as noted by Boyd, is interesting in connection with pathogenesis because Dowie-Dunn, Greenfield and Campbell have observed cases of Salmonella infection *in children* in the Cape Province where the clinical emphasis was that of colitis, the cytological picture of the stool that of bacillary dysentery and the evolution of the disease that of bacterial food poisoning in respect of mode of recovery, etc.

Escherichia in Generalized Infections and in Food Poisoning.

Colon bacillus septicæmias in man are pathological curiosities. Colon bacilli are common contaminants of *post-mortem* material and it would be best to base the proof that coli-bacillosis of calves is a true disease on evidence other than that obtained from the bacteriological examination of *post-mortem* materials. Special races of colon bacilli are in my experience sometimes responsible for mild food poisoning and it is possible that such may be concerned with coli-bacillosis of calves or indeed with epidemic infantile diarrhœas of children but the position at present is largely speculative, satisfactory proofs being lacking.

Dr. F. C. Minett (London) raised the question of food poisoning in man from staphylococcus products. Experiments were in hand to throw some light on the importance in this connection of staphylococci from cow's milk. Cats had been found unsuitable for this class of work; monkeys were more useful, though their susceptibility—by which was implied their capacity to vomit when fed with filtrates prepared from cultures of a known food-poisoning staphyloccoccus—was not high. It was essential, therefore, in this work, to use monkeys known by previous experiment to be susceptible. He had obtained gastro-intestinal disturbance with

vomiting in man and in monkeys, with culture filtrates of a number of hæmolytic staphylococcus strains from clinical cases of bovine mastitis and from "normal" milk. The conditions under which the "gastro-toxin" could be produced in milk, the nature of the substance and the practical importance of food poisoning from milk staphylococci were still conjectural.

Dr. C. Prausnitz (Ventnor, I.O.W.) said that at the last International Congress on Pædiatry in London Professor Bessau (Berlin) reported on the regular isolation by him in cases of infantile summer diarrhœa of specific strains of *B. coli* which were serologically uniform and highly virulent.

Mr. P. M. Short (London) also took part in the Discussion.

DISCUSSION *on* PATHOGENIC ANAEROBIC BACTERIA

Chairmen : Dr. E. J. Butler (London) and **Prof. M. Weinberg** (Paris).

Prof. M. Weinberg (Paris), **Opener :** *Récent progrès dans l'étude des microbes anaérobies.*

On peut diviser la période qui s'est écoulée depuis le moment mémorable de la découverte par Pasteur du premier microbe anaérobie jusqu'à nos jours en 2 époques : celle d'avant-guerre et celle d'après-guerre. Si la première a été marquée par la découverte des principaux microbes pathogènes et toxiques tels que : Vibrion Septique, B. tetanique, *B. perfringens*, *B. Novyi*, *B. botulinus* et *B. chauvœi*, la deuxième époque a enrichi nos connaissances en données très importantes non seulement sur les conditions physico-chimiques permettant la culture des microbes anaérobies, l'étude de leurs caractères biochimiques, mais aussi sur la structure de leurs antigènes, de leurs toxines ainsi que sur leur position dans la systématique.

Nous devons déplorer l'absence d'une discipline telle qu'elle existe chez les botanistes et les zoologistes pour la description des anaérobies nouvellement isolés ; la plupart des publications concernent des microbes insuffisamment décrits et souvent impossibles à classer de ce fait.

D'ailleurs, je dois dire en passant, que la classification des microbes anaérobies rencontre de grandes difficultés. On ne peut guère classer les microbes en se basant uniquement sur leurs caractères morphologiques. Nous ne pourrons faire une classification logique des microbes anaérobies qu'en tenant compte non seulement de la morphologie, mais aussi du pouvoir pathogène,

de la production de toxines ou de ferments, mais la chose ne sera possible qu'a l'avenir, lorsque tous les microbes auront été étudiés en même temps par les bactériologistes et par les chimistes rompus aux techniques très délicates de la chimie biologique.

Il a été décrit beaucoup d'espèces anaérobies nouvelles ; nous ne mentionnerons ici que les microbes les plus pathogènes et les plus toxiques : *B. œdematiens*, *B. histolyticus* (Weinberg et Séguin), *B. sordelli*, *B. hæmolyticus* (Record et Vawter), *B. gigas* (Zeissler), Bacille de l'osteomyelitis bulbarum de Kraneweld et des nouveaux types du *B. perfringens* (*B. agni* de Dalling, *B. paludis* de McEwen, Bacille D de Wilsdon et *B. ovitoxicus* de Bennetts. Parmi les microbes non pathogènes les plus intéressants signalons le *B. cellulosædissolvens* découvert par Mme. Khouvine et qui est le premier microbe cellulosolytique ayant été isolé certainement à l'état pur, chez l'homme.

Grâce aux progrès de la technique bactériologique, nous pouvons actuellement cultiver les microbes anaérobies sans extraction mécanique de l'air et sans emploi du mélange pyrogallol-potasse caustique. Il suffit pour cela d'utiliser le tube de Hall, la plaque de Fortner ou tout simplement des tubes de bouillon glucosé régénéré et additionné de quelque substance réductrice, comme acide pyruvique (A. Berthelot), sulfure de calcium (M. Nicolle), cystéine (Hosoya), etc. D'autre part, le procédé des géloses profondes (Liborius-Veillon) permet de pratiquer la séparation des microbes anaérobies.

Il ne faut pas oublier que si les microbes anaérobies les plus exigeants se développent, dans nos milieux de culture, en présence d'une très faible quantité d'Oxygène, ils demandent néanmoins une anaérobiose parfaite lorsqu'il s'agit de les isoler d'un produit pathologique quelconque.

On a beaucoup parlé de la possibilité de cultiver les microbes anaérobies en présence de l'air ; je crois qu'il faut dissiper un malentendu. Il ne s'agit pas, en réalité, de la culture en présence de l'air, mais d'une culture se développant dans un milieu dont la *surface libre seule* est en présence de l'air. Dans ce cas, les milieux de culture renferment des tissus d'origine animale ou végétale ou des substances chimiques capables d'éliminer l'Oxygène libre du milieu ensemencé. De telle sorte que, si le milieu de culture reste bien en contact par sa surface libre avec l'air atmosphérique, le microbe anaérobie, lui, reste protégé contre l'action nocive de l'Oxygène que renferme le milieu. D'autre part, les substances chimiques ajoutées au milieu de culture libèrent avec une grande facilité l'Hydrogène indispensable au déclanchement de la vie des microbes anaérobies.

Parmi les découvertes les plus intéressantes de ces derniers temps, il faut mentionner celles qui concernent l'existence de différentes races sérologiques, la dissociation des espèces microbiennes ainsi que la production des toxines multiples par une même espèce.

Vous savez que, jusqu'a ces derniers temps, lorsqu'on cherchait à établir le diagnostic d'une espèce microbienne par l'agglutination, on se servait d'un sérum agglutinant préparé au moyen d'une souche d'un microbe donné ; c'est surtout là la raison des nombreux échecs fournis par cette réaction. Dorénavant, on ne pourra affirmer que la séro-agglutination a donné une réaction négative que lorsque cette épreuve aura été faite soit avec différents sérums monovalents préparés avec les différentes souches de l'espèce microbienne donnée, soit avec un sérum polyvalent agglutinant toutes ces souches.

En effet, l'étude d'un grand nombre de *B. chauvœi* et de V. septique nous a fourni, à Mr. Mihailesco et à nous mêmes, les résultats suivants : toutes les souches du *B. chauvœi* étaient fortement agglutinées par le sérum anti-chauvoei, mais un certain nombre de ces souches ont donné des résultats différents lorsque nous les avons soumis à l'épreuve de la séro-agglutination pratiquée avec six sérums anti-V. septique préparés, chacun, avec une race différente de ce même anaérobie. Tel sérum anti-Vibrion septique donnait une réaction négative, tel autre, une réaction légèrement ou fortement positive. Vous voyez donc que si on avait pratiqué la réaction d'agglutination avec un seul sérum anti-Vibrion septique, on aurait pu conclure à tort que le *B. chauvœi* n'était pas agglutinable par le sérum anti-Vibrion septique.

Vous savez, depuis les recherches commencées par Arkwright et poursuivies par de nombreux chercheurs, que souvent le polymorphisme des colonies correspond à des variétés ou variantes et que, d'après leur aspect morphologique, elles représentent tantôt des races à caractères bio-chimiques différents, tantôt des races mobiles ou immobiles, pathogènes ou non pathogènes.

De grands progrès ont été réalisés dans la vaccination de l'homme et des animaux et dans l'hyperimmunisation des gros animaux producteurs de sérum grâce à l'emploi des toxines formolées (anatoxines de Ramon) et surtout grâce à l'enrobement des antigènes dans un produit lentement résorbable comme la lanoline. Ramon obtenu par cette téchnique, un excellent sérum anti-tétanique et nou mêmes avons obtenu, avec Mlle Guillaumie, très rapidement de sérums antigangréneux d'un titre élevé, en injectant des animau avec le mélange de toxines sèches et de microbes vivants centrifugé et repris par une petite quantité de toxine liquide, le tout enrob dans la lanoline.

Ajoutons que les travaux de Ramon et de ses collaborateurs on permis de réaliser la vaccination antitétanique. D'autre part, l sérothérapie de la gangrène gazeuse (Weinberg et ses collaborateur Bull, Vincent, etc.) a été découverte, celle du charbon symp tomatique (Leclainche et Vallée) et du botulisme (Velikanov a été mise au point.

Jusqu'à présent la réaction toxine-antitoxine était considérée comme un critérium certain ; deux faits nouveaux ont cependant

montré que cette réaction avait une valeur différente selon que l'espèce microbienne était mono- ou polytoxique.

En effet, l'étude des toxines multiples secretées par le *B. perfringens* et ses différentes variétés, comme le B. de Lamb Dysentery de Dalling, *B. paludis* de McEwen, Bacille D de Wilsdon et *B. ovitoxicus*, a montré que les substances toxiques secretées par la même espèce anaérobie se répartissent d'une façon inégale comme quantité et comme activité, dans les différents types microbiens appartenant à la même espèce. Aussi, peut-on trouver plusieurs anaérobies appartenant à la même espèce mais dont les composants toxiques prédominants sont tout à fait différents. Dans ces conditions, on comprend très bien qu'un sérum préparé avec une souche à prédominance d'un certain antigène toxique puisse être inactif vis-à-vis d'un autre type de la même espèce microbienne dont la toxine prédominante est complètement différente.

La réaction toxine-antitoxine ne pourra donc servir qu'au diagnostic d'un type mais non pas de l'espèce microbienne. Pour pouvoir faire le diagnostic de l'espèce, il faut préparer un sérum polyvalent actif vis-à-vis de tous les types d'une espèce microbienne donnée. Toutefois, comme lorsqu'on parle de la polyvalence d'un sérum, on pense à un sérum actif vis-à-vis de plusieurs espèces microbiennes différentes, nous avons proposé, pour éviter toute confusion, le terme de " holosérum " (holos, signifie en grec—total) pour désigner un sérum préparé contre tous les composants toxiques d'une même espèce. Nous avons réussi, avec Mlle Guillaumie, à préparer un holosérum anti-perfringens de titre antitoxique elévé vis-à-vis de tous les types du *B. perfringens.*

L'enseignement apporté par l'étude des différents types du *B. perfringens* parait bien s'appliquer également à d'autres espèces ; les faits observés au cours des recherches comparatives poursuivies d'une part, sur les différents types du *B. botulinus* et, d'autre part, sur le V. septique et le *B. chauvœi*, plaident en faveur de cette conception. Nous espérons que les " holo-sérums " (sérums anti-exo-endo-toxiques) de titre élevé vis-à-vis de toutes les toxines d'une espèce donnée permettront, non seulement de comprendre la cause de certains échecs de la sérothérapie, mais aussi de les réduire au minimum.

Prof. Ivan C. Hall (Denver, U.S.A.) : *The Present Status of Certain Recently Discovered Anaerobic Bacilli.*

The principal problems of anaerobic bacteriology may be divided into three categories, as follows : (1) problems dealing with the actual bio-physico-chemical mechanism of anaerobic growth ; (2) problems of nomenclature ; (3) problems concerning the specific properties and identities of certain species and their relation to pathogenic processes in man and animals, and to important industrial fermentation.

Only a few years ago those interested in anaerobic bacteriology had to give much attention to the development of technique, but technique is no longer a serious problem, because we now have a satisfactory set of procedures which enable us to examine any specimen for anaerobic bacteria, if not as quickly, at least as certainly, as for aerobic micro-organisms.

As for the mechanism of anaerobic growth, we are perhaps still far from a completely satisfactory explanation, but the recent progress made in this direction by McLeod, Fildes, and others, offers much hope.

As regards generic nomenclature, it is hoped that there may soon be a return to the sound basis of using solely morphologic and tinctorial criteria in the definition of genera, and that all of the sporulating rods, both aerobic and anaerobic, may be left in the single genus, *Bacillus*.

The third category of problems seems to me to be the most important of all to solve, and, fortunately, to offer the best hope of early solution. Our collection of more than 1,000 strains of anaerobic bacilli, brought together and maintained during the past 20 years, offers opportunities for studies of this kind. Among these problems to which our laboratory has recently contributed are the following :—

1. The identification of *B. œdematis sporogenes* (Sordelli) from Buenos Aires, and *C. œdematoides* (Humphrys, Melenny and Carp) from New York, with certain strains of the same species from cattle in Nevada, and from man in Colorado, and the valid re-naming of this species as *Bacillus sordellii* (Hall and Scott).
2. The identification of Rodella's Bacillus III and von Hibler's Bacillus IX as *B. paraputrificus* (Bienstock) and the frequent recognition of this species in the flora of the fæces of new-born infants and in other materials from human sources.
3. The discovery of a new species of pathogenic anaerobe, *B. difficilis* (Hall and O'Toole), in the fæces of new-born infants.
4. The discovery of new non-pathogenic species, *B. nonfermentans* (Hall), *B. subtumulis* (Hall) and *B. capitovalis* (Hall and Snyder).
5. The identification of von Hibler's Bacillus VI as *Bacillus carnis* (Klein).

Work in progress and nearly completed includes the following :—

1. A comparative study of many strains of *B. bifermentans* (Tissier and Martelly) and *B. centrosporogenes* (Hall) showing them to belong to the same species.
2. A study of about 100 strains of *B. welchii*, *B. paludis*, *B ovitoxicus*, and Dalling's lamb dysentery bacillus.

confirming Wilsdon's conception of their complex toxic spectra. It is suggested that the specific names should be retained for these as separate species rather than using letters to distinguish them as distinct " types " of *B. welchii.*

Prof. K. F. Meyer (San Francisco) [Communicated by Dr. H. Sommer (San Francisco)] : *Recent Studies on Botulism.*

During the past five years the following significant contributions have been made to the study of botulism :—

(1) Botulism—single and group-infections—has increased in the United States and Canada. 261 outbreaks (794 cases, 517 deaths) were chiefly due to inadequately preserved home-canned vegetables, several cases to soya bean mash or cheese fermented in the soil. Fish is the chief botulinogenic food in U.S.S.R. Tables were shown giving the incidence in U.S.A. and the regional world distribution of types.

(2) Fruit and vegetables preserved by freezing cause no botulism if carefully handled before freezing and consumed directly after thawing.

(3) Differences between *Cl. botulinum* and *Cl. parabotulinum* :—*Cl. parabotulinum*, the common organism in human outbreaks, especially in the United States, is strongly proteolytic, has very heat-resistant spores, is easily isolated, and produces Toxin Type A or B. *Cl. botulinum* (van Ermengem) is hardly proteolytic, has poorly heat-resistant spores, is difficult to isolate, and produces Toxin Type B, C, or D.

(4) Three strains recently obtained from U.S.S.R. resemble *Cl. botulinum*, but their toxin differs from the four known types.

(5) " Duck Sicknesss " in the Western United States is due to *Cl. botulinum* Type C, botulism of wild fowl in Australia to *Cl. botulinum* Type C β.

(6) The agglutination reactions of 160 strains were investigated by McClunn. The sub-groups established by Schoenholz and Meyer depend solely upon the thermo-labile antigen. All parabotulinum strains possessed the same thermo-stable antigen. No cross-agglutination occurred between *Cl. botulinum* and *Cl. parabotulinum.*

(7) Synthetic media :—Toxin is stated to be produced by *Cl. parabotulinum* in biuret-free media (Tani). Growth is stimulated by tryptophan and cystine (Burrows) or " Sporogenes vitamin " (Fildes).

(8) A new method is described for purifying botulinus toxin, giving a mouse M.L.D. of 4 to 8 $\times$ 10^{-9} gm.

(9) There is a high incidence of botulism in U.S.S.R. The death rate of 146 cases receiving antitoxin was 18, that of 76 untreated cases 93 per cent. (Velikanow and Kölesnikova).

Dr. J. Zeissler (Altona): *Wenig bekannte Anaeroben-Infektionen.*

Für das Verständnis der hier erörterten pathologischen Vorgänge ist unumgängliche Voraussetzung, dass der Arzt sich von der mit der Wirklichkeit nicht in Einklang stehenden Vorstellung frei macht, dass mehr oder weniger kurze Zeit nach dem Tode mit sogenannter Fäulnis einhergehend ein Durchwachsen der Leichen mit Keimen stattfände. Denn unter den bis jetzt von meinem pathologisch-anatomischen Kollegen Stoeckenius und mir bearbeiteten mehr als 500 Leichen war das Herzblut von etwa einem Drittel keimfrei Rund ein Drittel der Leichen enthielt in ihrem Herzblut ausschliesslich aerobe Keime und nur etwa ein Drittel anaerobe Bazillen. Diese Befunde sind nicht irgendwie unregelmässig oder zufällig. Ganz im Gegenteil haben wir in grossen Serien eine weitgehende Gesetzmässigkeit feststellen können.

1921 hat meine Mitarbeiterin Rassfeld das Herzblut von 400 Leichen bakteriologisch untersucht. Nach Abschluss dieser Untersuchungen stellten wir fest, dass die Prozentzahlen der Leichen mit Keimen im Herzblut und sogar die Prozentzahlen der gefundenen verschiedenen Arten von Keimen der Rassfeld'schen Untersuchungen ganz genau mit den entsprechenden Zahlen übereinstimmten, welche Strauch 1910 bei bakteriologischen Untersuchungen des Herzblutes von 2000 Leichen im Institut und unter Leitung des grossen Hamburger Pathologen Eugen Fraenkel gefunden hatte.

Von regelloser, ungehemmter Durchwucherung der Leichen in den ersten Tagen nach dem Tode mit Bakterien, insbesondere auch mit Anaerobiern, kann somit nicht die Rede sein.

Zu allem Überfluss haben wir dann noch bei 12 Leichen je 10 ccm einer frischen üppigen Kultur des *Vibrion septique*, eines besonders leicht und rasch wachsenden Gasödembazillus, in die Muskulatur des Thorax und eines Oberschenkels eingespritzt 12 Stunden bis 3 Tage vor der Obduktion. Nie konnte in diesen Fällen bei der späteren Obduktion der in die Thoraxwand und in den Oberschenkel eingespritzte *Vibrion septique* aus dem Herzblut wieder gewonnen werden. Das ist nur ein einziges Mal gelungen nach Einspritzung in die Leber unter Druck.—Hier ist jedoch nicht ein Durchwachsen des Körpers nötig, um die eingespritzten Keime im Herzen wieder zu finden, sondern es steht bei solcher Versuchsanordnung der Weg durch die Vena cava offen.

Unsere mikroskopischen Präparate von Gewebsschnitten zeigen, dass sich die Ausbreitung der Keime im Körper durch die verschiedenen Teile und Organe noch in der Leiche streng der Zirkulation des Blutes folgend nachweisen lässt, und das beweist, dass die Ausbreitung der Keime durch die verschiedenen Teile und Organe des Körpers ein vitaler Vorgang ist und mit dem Stillstand der Blutzirkulation ebenfalls zum Stillstand kommt. Nur eine Vermehrung der Keime an all den Stellen, wohin die Blutzirkulation sie intra vitam geschwemmt hat, kann nach dem Tode noch stattfinden.

Von dieser Regel werden Sie auch sogleich eine sehr lehrreiche Ausnahme sehen, ein direktes Überwachsen von Keimen aus einer verätzten Magenwand in die ihr direkt anliegende ebenfalls verätzte Milzkapsel.—Eine Verätzung der Gewebe war nötig, um das direkte Durchwandern der Keime von einem Organ zum anderen zu ermöglichen.

Die Fälle, die ich Ihnen vorführen werde, haben als gemeinsames Charakteristicum die Plötzlichkeit des Todes. Bei mehreren von ihnen wurde eine Vergiftung durch ein chemisches Gift angenommen und gerichtliche Obduktion angeordnet, bei anderen krankhafte Zustände als Todesursache diagnostiziert, die schon längere Zeit bestanden hatten und für sich allein den Eintritt des Todes nicht ausreichend rechtfertigten. Ein Fall war klinisch als Milzbrand diagnostiziert, weil zehn Tage vor dem Tode der Kranke tatsächlich zwei kleine bakteriologisch verifizierte Milzbrandpusteln am Halse gehabt hatte, die aber inzwischen abgeheilt waren.

Doch nicht nur die Plötzlichkeit des Todes ist ein gemeinsames Charakteristicum unserer Fälle, sondern ebenso auch ihre Ätiologie: stark toxische Gasödembazillen, meist der Novysche Bac. des malignen Ödems, oder der *B. gigas*, seltener der *Vibrion septique* und der Fraenkelsche Gasbacillus (*B. Welchii*).

Auch das Zustandekommen der Infektion zeigt bei unseren Fällen weitgehende Übereinstimmung. An histologischen Schnitten werde ich Ihnen das bakterioskopisch demonstrieren.

Unsere Fälle sind somit Fälle von Mors subita, hervorgerufen durch Infektionen mit hoch toxischen Gasödembazillen auf eine bis jetzt noch wenig oder garnicht bekannte Weise.

Meine Ausführungen werden an Wahrscheinlichkeit gewinnen, wenn ich auf eine ganz analoge Krankheit hinweise, seit Jahrzehnten den Veterinären unter den Namen "Bradsot," "Braxy" geläufig. Bradsot bedeutet "Mors subita."—Auch diese "Mors subita" der Schafe wird durch dieselben hoch toxischen Gasödembazillen erzeugt, die wir bei unseren Fällen von "Mors subita" des Menschen als Erreger festgestellt haben.

Die vorgeführten Bilder demonstrieren das Eindringen von Gasödembazillen und ihre Verbreitung durch den Blutkreislauf im Körper vom *Darm* aus durch ein kleines *tuberkulöses Jejunalulcus*, vom *Darm* aus durch ein *leukämisches Ulcus* des Colons, durch eine *entzündete Gallenblase*, durch eine durch 96 *prozent Alkohol* und durch *Salzsäure verätzte Magenwand*, von einem *malignen Ödem der Harnblase* aus, durch *Aspiration* von Mageninhalt von der *Lunge* aus bei Thrombosekrankheit, von *Malignem Ödem der Nasenschleimhaut* aus (2 Fälle), von *minimaler Verletzung* der *Lippe* und *Nase* aus, von einem *Furunkel* am Oberschenkel aus, von einem alten *Ulcus cruris*, von einer abgeheilten *Anthrax-Pustel* aus, also stets von *Defekten* der schützenden *Haut-* und *Schleimhautdecken* des Körpers aus, genau so, wie es schon 1921 Rassfeld für das Eindringen von anaeroben Bazillen in den menschlichen Körper nachgewiesen hat.

Alle diese *plötzlichen Todesfälle* müssen zurückgeführt werden auf die *Giftwirkung der Gasödembazillen, welche an den genannten Einbruchstellen* in den Körper eingedrungen sind.

Die klinischen Diagnosen unserer Fälle von "Mors subita" des Menschen — Sepsis, Darmtuberkulose, Urämie, Vergiftung durch konzentrierten Alkohol, Vergiftung durch Salzsäure, plötzlicher Tod bei Cholecystitis, Thrombosekranheit, alte chronische Streptokokkensepsis, artifizieller Abort oder chemische Vergiftung, schwer erklärliche Todesfälle bei geringfügigen Verletzungen, atypische Furunkel, Ulcus cruris, gelegentlich sogar Anthrax zeigen uns, bei welchen *klinischen Krankheitssymptomen* an *Intoxikationen durch die Toxine der Gasödembazillen gedacht werden muss.*

Dr. R. Prigge (Frankfurt a.M.): *Experimentelle Untersuchungen über die serologischen Eigenschaften des Fraenkel'schen Gasbazillus* (B. perfringens).

Die Giftwirkungen des Fraenkel'schen Gasbazillus (*B. perfringens*, Typus A der Welch-Gruppe) werden vorwiegend dem Hämotoxin zugesprochen. "Toxin" und "Hämolysin" gelten als identisch und werden als "Alpha-Toxin" oder "W-Faktor" den bei anderen Typen der "Welch-Gruppe" gefundenen Giften gegenübergestellt. Ich fand indessen, dass bei Verwendung von *Peptonen verschiedener Herkunft* Unterschiede in der Giftwirkung der Kulturfiltrate des Gasbazillus vorkommen: einige töteten die Versuchstiere unter deutlicher, andere ohne nennenswerte Hämolyseerscheinungen. Also musste entweder die Hämolysinbildung variabel sein oder der verwendete Stamm, A 52 P, aus verschiedenen Toxinbildnern bestehen. Herr *Zeissler* zerlegte ihn mikromanipulatorisch in eine Komponente mit *runder* und eine mit *gezackter* Kolonieform. Bei Züchtung dieser Komponenten auf gleichen Nährböden waren keine Unterschiede in der Relation Toxin: Hämolysin feststellbar. Die beobachteten Schwankungen waren also wahrscheinlich durch Nährbodenunterschiede verursacht. Auch Neutralisationsversuche mit verschiedenen Seren ergaben *keinerlei quantitative Unterschiede* zwischen den Toxinen der beiden Komponenten (Tab. 1).

TABELLE I.

	Bei Verwendung verschiedener Toxine ermittelte Titer				
	des dänischen Perfringens Standard-Serums: AE/ccm	des deutschen Perfringens Standard-Serums: AE/ccm	des englischen Perfringens Standard-Serums: AE/ccm	des monovalenten Perfringens Serums Nr. 1980[1] AE/ccm	des monovalenten Perfringens Serums Nr. 1987[2] AE/ccm
Toxin Prg 2L ..	20	62,5	20	10,6	6,0
Toxin Prg 187 ..	19	62,5	19	13	7,6
Toxin Prg 253 ..	19	62,5	19	13	7,6

[1] mit der "runden" Form hergestellt.
[2] mit der "gezackten" Form hergestellt.

Abweichende Ergebnisse zeigte dagegen Toxin Prg 2 L, das in England mit ganz anderen Peptonen hergestellt und mir von Herrn Dr. *Hartley* unter der Bezeichnung " Welchpool " freundlicherweise übergeben wurde. Es lieferte teilweise geringere, teilweise höhere Titer als die Gifte Prg 187 und Prg 253. Zur Aufklärung dieser seit längerem bekannten Unstimmigkeiten wurde eine *genaue Analyse der geprüften Perfringensgifte* vorgenommen. Prg 2 L war erheblich stärker hämolytisch als das aus der " runden " Form gewonnene Prg 187 (Tabelle II).

TABELLE II.

	L† 50 (0,2 AE)	dl	L H (0,2 AE)	dhm	dhm/ dl	LH/ dhm	L† 50/ LH
Prg 2L . .	2,6 mg	0,3 mg	5,0 mg	0,5 mg	1,7	10	$\sim\frac{1}{2}$
Prg 187 . .	1,35 mg	0,15 mg	5,0 mg	2,5 mg	17	2	$\sim\frac{1}{4}$

Folgende weitere Schlüsse ergeben sich aus dieser Tabelle :

(1) Der Quotient der in vitro hämolytischen Dosis durch die für Mäuse intravenös tödliche Dosis beträgt für Prg 2 L 1,7, für Prg 187 dagegen 17,0. *Die Gifte müssen also neben dem hämolytisch wirkenden Alpha-Toxin eine weitere toxische Substanz enthalten, die wir Zeta-Toxin nennen.* Die Vergiftungserscheinungen beim *Tier* kommen durch das Zusammenwirken beider Toxine zustande. Hiermit stimmt die Beobachtung überein, dass im Tierversuch ein geringer Toxinüberschuss von Prg 2L stärkere intravitale Hämolyse verursacht als die adäquate Giftmenge von Prg 187.

(2) Intravenöse Injektion von 0,2 ccm einer 1 : 50 - Verdünnung des amerikanischen Standardserums (= 0,2 AE) mit 2,6 mg Prg 2L (= L † 50) tötet etwa 50 prozent der behandelten weissen Mäuse. Aber erst eine Mischung der gleichen Serummenge mit 5,0 mg Gift (= LH) entfaltet eine in vitro erkennbare hämolytische Wirkung. — Das Alpha-Toxin spielt also beim Tod der mit 0,2 AE + L † 50 vergifteten Tiere nur eine untergeordnete Rolle. — Eine Mischung von 0,2 ccm einer 1 : 10,6-Verdünnung des Serums 1980 mit 2,6 mg Prg 2L tötet ebenfalls 50 prozent der Versuchstiere, jedoch *bewirkt bereits eine Mischung von* 2,0 *mg Gift mit dieser Serummenge deutliche Hämolyse.* 2,6 mg Gift + 0,2 ccm Serum 1980 (1:10,6) enthält also mehr freies Alpha-Toxin als eine Mischung der gleichen Toxindosis mit 0,2 ccm des amerikanischen Standardserums (1:50), tötet jedoch den gleichen Prozentsatz Tiere. Offenbar ist also dem Alpha-Toxin im zweiten Fall sehr viel höhere, dem Zeta-Toxin dagegen geringere Bedeutung für den Tod der Tiere beizumessen als im ersten Fall.

(3) Der Unterschied zwischen den LH-Dosen, die mit dem amerikanischen Standardserum und dem Serum 1980 gefunden wurden (5,0 und 2,0 mg Prg 2L), zeigt, dass eine bestimmte Menge

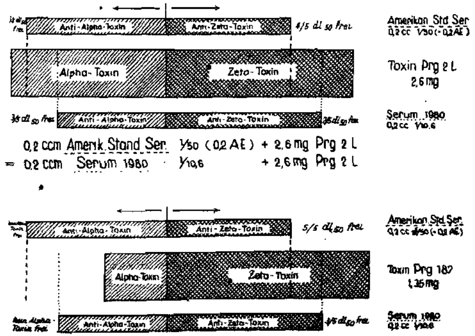
des letzteren weniger Anti-Hämotoxin enthält als eine im Tierversuch "äquivalente" Menge des ersteren. Man muss also schliessen, dass umgekehrt Serum 1980 relativ mehr Anti-Zeta-Toxin enthält als das amerikanische Standardserum, und dass der Überschuss an freiem Alpha-Toxin, den ein Gemisch des Serums 1980 mit 2,6 mg Prg 2L enthält, durch stärkere Neutralisation des Zeta-Toxins ausgeglichen ist. Hiermit stimmt überein, dass bei intrakutaner Injektion an ein und demselben weissen Meerschweinchen Serum 1980 10 prozent mehr Prg 2L neutralisierte als eine "äquivalente" Menge des amerikanischen Standardserums. Aus dieser Umkehrung der Neutralisationsverhältnisse muss aber nicht gefolgert werden, dass das Alpha-Toxin an der Entstehung der Hautveränderungen *überhaupt* keinen Anteil hätte.

(4) Die L†-Dosis von Prg 2L repräsentiert mehr als das Fünffache der dhm. Die L†-Dosis von Prg 187 entspricht dagegen nur etwa der Hälfte einer dhm. Der Hämotoxingehalt dieses Giftes spielt also bei der Antitoxintitration eine *wesentlich geringere Rolle.*

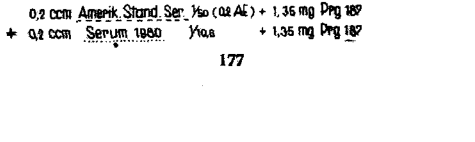
(5) Auf Grund der bisher erwähnten Feststellungen können die Unterschiede zwischen den mit verschiedenen Giften im intravenösen Schutzversuch ermittelten Antitoxin-Titern verständlich gemacht werden. Das Diagramm (Tabelle 3) soll in *schematischer* Weise, aber *ohne Berücksichtigung der genauen quantitativen Verhältnisse veranschaulichen, wie sich die Wirkungen des nicht neutralisierten Alpha und Zeta-Toxins summieren.*

DIAGRAMM

I



II



Das Diagramm zeigt, dass 0,2 ccm einer 1:10,6-Verdünnung von Serum 1980 bei Mischung mit 2,6 mg Prg 2L das genaue Äquivalent von 0,2 AE darstellt, während die gleiche Serummenge bei Mischung mit 1,35 mg Prg 187 viel wirksamer ist. *Dem Serum* 1980 *wird also im zweiten Fall ein höherer Titer zugesprochen als im ersten Fall.* Umgekehrt würde ein Serum mit relativ höherem Anti-Hämotoxin-Gehalt mit Prg 187 *geringeren* Titer zeigen als mit Prg 2L. Dieser Fall dürfte z.B. bei dem dänischen und dem englischen Standard-Serum vorliegen.

(6) Die pathologisch-anatomischen Befunde von Herrn *Zeissler* mit Toxin Prg 187 sprechen dafür, dass die hämolytischen Prozesse bei der Ätiologie des Gasbrandes wesentlich geringere Bedeutung besitzen als die toxischen Wirkungen. Bei der Serumproduktion erscheint es daher als wichtigste Aufgabe, Sera mit einem möglichst hohen Gehalt an Anti-Zeta-Toxin zu gewinnen. *Es ist also zu fordern, dass die Wertbemessung der Perfringens-Sera nur mit* hämotoxinarmen Giften durchgeführt wird, weil nur mit solchen Giften eine befriedigende Bestimmung ihres *Anti-Zeta-Toxin-Titers im Schutzversuch möglich ist.* Wenn sich ein gewisser *Anti-Hämotoxin-Gehalt* als notwendig erweisen sollte, *muss er unabhängig vom Anti-Zeta-Toxin-Titer ermittelt werden. Dass zur Herstellung der Testgifte nur mikromanipulierte Stämme verwendet werden dürfen, sollte eine Selbstverständlichkeit sein.*

(7) Bei Prg 2L entsprechen einer LH-Dosis 10 dhm, bei Prg 187 dagegen nur 2! Prg 187 enthält also das *Hämotoxin in einer sehr toxoidreichen Form.* Hierdurch wird es verständlich, dass trotz der sehr beträchtlichen Differenz im absoluten Hämotoxingehalt die auf eine L†50-Dosis entfallenden "*Hämo-Antigen-Mengen*" nur verhältnismässig kleine Unterschiede aufweisen. Daher kann man auch mit Giften von der Konstitution des Prg 187 Sera gewinnen, welche einen gewissen Gehalt an Anti-Hämotoxin besitzen.

Die ausführliche Veröffentlichung dieses Vortrages erfolgt in der Zeitschrift für Immunitäts-Forschung" Bd. 89; H.6.

Dr. J. Zeissler (Altona).

Zu dem Vortrage des Herrn Prigge bemerke ich ergänzend, dass mein Assistent, Herr Dr. Goertzen, Lösungen des uns hierfür von Prigge zur Verfügung gestellten "Perfringenstoxins 187," von ihm hergestellt aus der mehrfach mikromanipulierten "runden" Komponente des Stammes "Perfringens A 52 P" Kaninchen, Meerschweinchen und Mäusen intravenös injiziert hat.

Die Versuchstiere erhielten fallende Toxinmengen und zwar

die Mäuse	1	bis	35 tötliche Minimaldosen für Mäuse,
die Meerschweinchen	10	bis 1000	,, ,,
die Kaninchen	500	bis 2500	,, ,,

Alle (5) Kaninchen starben akut innerhalb weniger Minuten. Die Meerschweinchen, welche 40 und mehr tötliche Minimaldosen für Mäuse erhalten hatten, starben je nach der Toxinmenge innerhalb 1 bis 78 Stunden, die Meerschweinchen mit geringeren Toxindosen blieben am Leben. Die Mäuse starben alle innerhalb 2 bis 21 Stunden.—Anzeichen von intravitaler Hämolyse boten nur Mäuse, und zwar nur diejenigen, welche 6 Stunden und länger lebten.

Diese Beobachtung spricht für die Richtigkeit der Anschauung Prigges, dass für den Tod der Tiere das *Zeta*toxin der wesentliche Faktor ist.

Herr Prigge hat seine Toxinstudien mit Bazillenstämmen ausgeführt, welche in meinem Institut von meiner Mitarbeiterin Rassfeld mit dem Peterfi-Zeiss'schen Mikromanipulator mehrfach mikromanipuliert worden sind. Vor dem Mikromanipulieren wurden diese Bazillenstämme—manchmal in mehrmonatiger Arbeit—auf der Oberfläche von Traubenzuckerblutagarplatten und in Leberbouillon abwechselnd gezüchtet, bis sich aus ihnen Komponenten abgespalten hatten, welche einerseits in dicken, runden, knopfförmigen, andrerseits in flachen Kolonien mit zackigen Ausläufern wuchsen. Letztere enthielten vielfach lange Bazillenfäden.

Für Toxinstudien an anaeroben Bazillen ist heute die vorherige Bearbeitung der zu benutzenden Bazillenstämme mit dem Mikromanipulator unumgängliche Voraussetzung.

Mr. T. J. Bosworth (Cambridge): *The Rôle of* Clostridium welchii *and other Anaerobes in Disease of Sheep.*

Interest in the common pathogenic anaerobes is largely centred at present on their relationship to disease in animals. *Cl. welchii* is of much greater importance in this connection than was previously suspected. Many rapidly fatal cases of illness in sheep are due to intoxication by the toxins produced in the intestine by various types of this organism, which differ from one another in the antigenic character of their toxins (Wilsdon). The relationship of these organisms to one another and to the classical type of *Cl. welchii* (*B. perfringens*) is now understood. Thus it has become possible to immunize animals against the entire group. Application of this procedure to man might be considered. Type A is not the only member widely distributed in nature. Although the other types which are more potent toxin producers have not been isolated from human lesions, the possibility of their gaining access to the body through wounds or otherwise cannot be disregarded.

The conditions favouring the onset of entero-toxæmia in sheep require further investigation. Besides its scientific interest, this problem has considerable economic importance, since probably *Cl. welchii* causes a greater wastage of animal life in this country than any other anaerobe.

Other important diseases of this class met with in Great Britain are Blackleg and Braxy. The ætiology of the former is still controversial. The weight of opinion is to the effect that it is a specific disease caused by *Cl. chauvœi.* Quite apart from other considerations, the continued success of the use of preventive vaccines prepared from this particular organism affords strong confirmatory evidence in favour of this view.

Miss G. R. Borthwick (Bristol) : *Pathogenic Anaerobic Bacteria.*

(1) *Factors influencing the occurrence of infection from the alimentary tract.*

In infection of guinea-pigs by the enteral administration of *Vibrion septique,* the hydrogen-ion concentration of the gastro-intestinal contents and the local application of cold to the alimentary mucosa have little effect. Stasis and generalized chilling, on the other hand, predispose to infection. *B. ovitoxicus* fed to sheep produces infection only in association with reduced peristalsis and an alkaline reaction of the gastric contents.

Toxæmia is not produced by the enteral administration of *Vibrion septique* toxin to guinea-pigs and *B. ovitoxicus* toxin to sheep under any of the conditions studied in the production of infection.

(2) *The toxins of the* B. welchii *group.*

Observations on the toxins of the *B. welchii* group confirm the classification of *B. welchii* strains into the now accepted types, A, B, C, and D, according to their serological characters. Except for the similarity between the toxins of type C and *B. œdematiens,* there is no relationship between these toxins, A, B, C and D, and the toxins of certain other members of the anaerobic group. The monovalent character (A) of commercial *B. welchii* antitoxin was demonstrated. A general parallelism between the antitoxic and anti-infective properties of *B. welchii* sera was shown. A striking exception, however, is that type A and commercial antisera protect against infection by type D organisms.

Dr. H. A. Gins (Berlin) [Communicated by Prof. J. Fortner (Berlin)] : *Pathogene anaerobe Bakterien.*

Auf Grund unserer Erfahrungen in den letzten Jahren lassen sich einstweilen folgende Schlüsse über die mögliche Pathogenität der *sporenlosen* anaeroben Bakterien ziehen :

(1) Unter den mehr als 25 Arten nicht versporender Anaerobier, die wir zum ersten Mal reingezüchtet haben, finden sich mehrere Arten, bei denen kaum mehr ein Zweifel an der Pathogenität bestehen kann.

(2) Die Leptothrix Gi XIX z. B. ist durch Agglutination und spezifisch bakterizide Wirkung des Patientenserums als Ursache eines subphrenischen Abszesses erkannt worden. Die therapeutische Verwendung der Reinkultur zur Autovaccine war erfolgreich.

(3) Fortschreitende Knotenbildungen mit Abszedierung, die unter dem klinischen Bild der Aktinomykose verliefen, ergaben mehrmals bakteriologisch die alleinige Anwesenheit typischer Vertreter der anaeroben Mundflora, waren aber frei von Aktinomyzeten.

(4) Als Erreger einer klinisch sicheren Aktinomykose wurde eine Aktinomyzesart isoliert, die durch die strenge Anaerobiose und auch morphologisch keine Aehnlichkeit mit dem Aktinomyces Boström hat. *Die einheitliche Aetiologie der klinischen Aktinomykose muss daher angezweifelt werden.*

(5) Nachdem einzelne dieser Anaeroben als pathogen erkannt sind, darf die massenhafte Anwesenheit dieser Formen bei der Zahnkaries und bei der Paradentose (Alveolarpyorrhoe) nicht länger als bedeutungslos angesehen werden.

(6) Die Erforschung des Einflusses der erwähnten Bakteriengruppe auf diese Erkrankungen ist eine der wichtigsten Aufgaben der Mikrobiologie, weil sie Aufschluss darüber geben wird, ob eine systematische Prophylaxe der Zahn- und Mundkrankheiten möglich ist.

Dr. J. M. Alston (London): *Human Infections with Organisms resembling Bang's Necrosis Bacillus.*

In addition to older references, accounts have been given recently by Shaw (1933), Shaw & Bigger (1934) and Franklin (1933), of human infections by a gram-negative, pleomorphic, anaerobic organism which they identify with *B. necrophorus* (*Actinomyces necrophorus, Fusiformis necrophorus*, Bang's necrosis bacillus). Lemierre (1936) has reported cases of septicaemia in human beings due to a similar organism which he names, after French workers, *B. funduliformis*. The present speaker is reporting 9 cases which he has observed in London since October, 1933, and which he considers due to *B. necrophorus*. The general nature of the infections was abscess formation, either single or multiple, and the pus had a very heavy, cheese-like odour. Microscopically, the organism appeared in the pus as a small Gram-negative bacillus like Pfeiffer's bacillus or sometimes longer, and other organisms, especially non-hæmolytic streptococci, were often present. The bacillus was isolated in pure culture in six cases and apparently in mixed culture in two others. Films made from cultures showed much greater pleomorphism as regards length, thickness and curving of the organism than films prepared from pus. Pure cultures had a cheesy odour similar to the pus. Two strains of the bacillus were shown to produce slow abscess formation in rabbits and mice. In two of the human cases the primary focus appeared to be the female pelvic organs, in one case the throat, in five empyema was the chief lesion with no evident site of entry, and in one case either mediastinitis or pericarditis was the earliest lesion found. All, except three of the empyema cases, died.

Dr. F. Patočka (Prague): *A Case of Mycosis of the Buccal Mucous Membrane caused by a strictly Anaerobic Thread-shaped Micro-organism of the* Leptothrix *Type.*

A few cases of disease due to thread-shaped bacteria are mentioned in the medical literature but only in rare cases could cultures of the causal organisms be obtained. These bacteria are regarded by some authors as "incomplete pathogénic bacteria." Obertel and I observed an affection of the mucous membrane in the mouth under the tip of the tongue in a man who was otherwise healthy. The lesion, which at first appeared harmless, was a rather adherent pseudomembranous lesion; some signs of inflammation developed at a later stage. Any other cause of the lesion was excluded. In the pseudomembrane thread-shaped bacteria of varying length, some of them arranged in chains, were found, which undoubtedly belonged to the Leptotricheæ. Under strictly anaerobic conditions we succeeded in growing two kinds of Leptothrix on 15 per cent. rabbit blood agar plates. As they could not be identified with any known bacterial cultures they were called Leptothrix A and Leptothrix B. Leptothrix A grew on the surface of the blood agar plate in fine linear colonies, which slowly developed into fur. In liver broth with serum and blood many fine flakes formed, without turbidity, but with considerable gas formation. Microscopically the growth consisted of very long Gram-labile threads, some of which formed chains. Leptothrix B formed round strawberry-coloured colonies with yellowish centres. None of the fluid culture media showed any special characteristics, but the cultures grew well. Microscopically, the culture consisted of small threads with numerous granules. Neither strain was pathogenic for animals, but possibly, owing to the prolonged culture, their virulence had disappeared. Leptothrix A. inoculated intracutaneously in man, caused a temporary local inflammation. Both produced antibodies in animals. Leptothrix A and Leptothrix B are probably new saphrophytic organisms, which are well suited to the human body and accidentally become pathogenic. A cancer developed in the original lesion after 3½ years, and led to a fatal issue.

Dr. D. W. Henderson (Elstree, England), said that the present haphazard method of classifying the spore-bearing anaerobes led to an ever increasing number of species names; it must inevitably bring endless confusion.

The method of serological analysis was suggested as a rational means of accumulating data upon which a sound basis for classification could be founded. It had been established that the specificity of serological reactions was determined by the chemical structure of the antigen and a knowledge of this in relation to micro-organisms would show a true relationship of one organism to another.

Dr. E. J. Butler (London) and **Prof. M. Weinberg** (Paris) summed up the discussion.

SECTION 4

ECONOMIC BACTERIOLOGY. SOIL, DAIRYING AND INDUSTRIAL MICROBIOLOGY.

SUBSECTION 1—DAIRY MICROBIOLOGY

DISCUSSION *on* THE SIGNIFICANCE OF THE NUMBER AND TYPES OF BACTERIA IN MILK, INCLUDING THERMODURIC AND THERMOPHILIC ORGANISMS. THE NEED FOR ADOPTING UNIFORM METHODS

Chairman : Prof. R. S. Breed (Geneva, U.S.A.).

Prof. R. S. Breed (Geneva, U.S.A.), **Joint Opener.**

The foundation of a good milk supply is good milk. This must come from a healthy cow, and it is also desirable that it should come from a well-constructed, clean stable. As the essential qualities of a good milk are not always evident to the person who consumes it, it becomes necessary to establish official inspection and control procedures.

The primary purposes of microbiological control are to protect the milk against contamination with dangerous disease germs, and to prevent the growth of excessive numbers of micro-organisms. While the first purpose has been in the minds of many public health workers in North America during the development of the work on control of market milk, in recent years it has become increasingly clear that the reduction of the total number of bacteria present in milk, together with pasteurization, accomplishes both purposes.

It has been realized for a long time that the present standard agar of the American Public Health Association is not an entirely satisfactory medium to use in endeavouring to bring about a reduction in the total flora, inasmuch as certain fastidious types of bacteria do not find this a suitable agar on which to grow. Moreover, 37°C. is a poorly selected temperature for incubation ; it is too warm for many types of bacteria that should be found and eliminated from our milk supplies, and at the same time too cold to permit growth of truly thermophilic types. It has been suggested[1] that incubation for two days at 32°C. and, if necessary, incubation for one additional day at 45°C. would reveal all important groups of bacteria provided the agar used is of a satisfactory composition.

At the present time in America studies are under way to determine whether an agar containing digested casein (Tryptone, Difco), glucose and skim milk[2] is a satisfactory agar for the growth of all types of bacteria which are significant, primary incubation being for two days at 32°C.[3]

A brief history of the use that has been made of approved and standardized methods for controlling the bacteriological conditions of market milk supplies in the U.S.A. may be of interest. The

[1] H. N. Parker, Jacksonville, Florida. Private communication.
[2] Bowers, C. S., and Hucker, G. J., *Amer. J. Pub. Health*, 1936, **26**, 350–353.
[3] Yale, M. W., and Pederson, C. S., *Amer. J. Pub. Health*, 1934, **24**, 477–484 ; 1936, **26**, 344–349.

standardization of these procedures was undertaken between 1905 and 1910 by members of the American Public Health Association in a purely voluntary and non-official capacity. So effective and satisfactory has the work of this international association been that their so-called Standard Methods of Milk Analysis, the first edition of which appeared in 1910, has appeared in successive editions until the present is the sixth edition. It was issued in 1933 and a new (7th) edition is in process of preparation. This work has been officially recognized in many states of the Union, in Canada, Cuba and Mexico by laws and regulations that require that the laboratory methods of the American Public Health Association be used in milk control work in the areas covered by these laws and regulations.

The foundation technique that has been used in North America has been the agar plate count and the purpose behind its use has been to reduce the total bacterial population to a minimum. Throughout, we have had those who have argued that the bacterial counts obtained by this method were so inaccurate as to be valueless and others who have argued for the use of methods designed to find specific types of bacteria such as the colon, tubercle, undulant fever, septic sore throat, anaerobic gas formers, or other undesirable types of bacteria.

However, time has demonstrated the wisdom of the various successive committees of the A. P. H. A. who have consistently urged that the agar plate count and other methods of estimating the total bacterial population, such as the direct microscopic technique approved as a standard procedure in 1916 and the methylene blue reduction technique approved as a standard procedure, in 1923, be used as a means of keeping the total bacteria content of milk at the minimum. Each of these methods has found its own field with us and all are being developed and used to an increasing extent. No one of the three methods yields absolutely accurate counts, and there being no accurate standard for comparison, nobody knows to this day which method of estimating the bacterial population of milk is the most accurate.

In America, the methylene blue technique is regarded as especially valuable as an introductory technique for controlling poorer quality raw milk supplies where an intelligent but not technically trained personnel is available. The results secured from the direct microscopic technique are more useful than those secured from the methylene blue technique because more information regarding the nature and cause of high counts is secured and nothing stainable is overlooked; but it requires a more technically trained personnel.

The agar plate technique in spite of its obvious limitations, remains the basis of our official control of bottled milk as delivered to the consumer. In spite of statements that have been published that imply a contrary condition, the use of this technique for this purpose shows a steady increase. It also is used in the U.S.A. for the exami-

ination of an even larger number of raw milk samples where premiums are paid to Grade A dairymen who deliver milk with an agar plate count less than 10,000 per cc.

The rapidity of the development of the use of the methylene blue technique for the control of raw milk supplies has deceived some into thinking that it was replacing the agar plate and direct microscopic technique.

TABLE 1.—STATISTICS SHOWING THE NUMBER OF SAMPLES OF MILK ANALYZED BY VARIOUS LABORATORY METHODS IN MILK CONTROL LABORATORIES IN THE UNITED STATES AND CANADA.

Year	Number laboratories • included in report	Technique Used		
		Agar Plate	Direct Microscopic	Methylene Blue Reduction
1910	50+	Less than 20,000	None	None
1925	152*	200,000+	30,000+	10,000+
	89**	1,000,000+	100,000+	100,000+
1929–30	342*	568,000	296,500	151,000
	125**	2,104,000	751,400	584,000
1935–36†	354*	831,600	117,100	282,500
	134**	1,891,000	815,000	1,541,700°

\+ This is a liberal estimate.
* State, Provincial and Municipal Laboratories of the U.S.A. and Canada.
** Industrial Laboratories.
° More than half the work was done by one milk company.
† Statistics for 1935–36 incomplete, returns from laboratories still being received, October, 1936.

It is difficult to get accurate statistics to indicate the development that is taking place. If the figures given in Table 1 were complete they would show an increasing use of all three of the commonly used methods for estimating the total bacterial count; each being used in its own special field. Nevertheless, the difficult economic condition that has existed since 1929 has lessened the amount of laboratory work done in many places. Perhaps the largest cut has occurred in the New York City area where the number of analyses made to secure agar plate counts used as a basis of payment of premiums to Grade A dairymen has decreased greatly because of lessened sales of Grade A milk.

Dr. A. T. R. Mattick (Shinfield, Reading), **Joint Opener.**

An enormous literature now exists upon the enumeration of bacteria in milk.

The plate count method is commonly used, and many workers have introduced improvements—in details of technique, media, aids to correct enumeration of colonies and alterations in incubation temperature. All these things have arisen from a widespread

dissatisfaction with the results secured—a dissatisfaction which has steadily increased with the more general application of statistical methods to the biological sciences.

In some kinds of milk the reproducibility of the plate count is very poor, for one reason owing to the uneven distribution of bacteria in milk. With some milks fantastic differences from worker to worker may be found, and it is a matter of grave doubt whether it is possible, even if desirable, to classify milk on any but the broadest lines by the use of the plate count. For raw milk of the highest quality the plate count may yield results on which some reliance may be placed, but for all other types of milk there is little doubt that apparent differences between the figures for one milk and those for another should be accepted with the greatest reserve; in grading, for whatever purpose, wide differences between grades must necessarily be fixed. That it is unjust to condemn a milk supply upon the result of one examination is well known and should be emphasized. Even with the best class of milk it is only possible to secure moderately concordant results if all samples are stored and transported under identical conditions and examined at the same age. Owing to the determining influence of temperature upon bacterial growth in most types of milk—to mention the most important factor—uniformity of treatment before examination is essential. For advisory purposes the plate count, together with such tests as the Breed count, is of considerable value. For assessing the quality of milk containing more than small numbers of bacteria, in certain types of milk, accumulated evidence points to the necessity for reconsidering the use of the plate count.

Grading of milk for public health purposes and the determination of the commercial properties of milk are not quite the same thing, and in this connection the temperature at which the control test is carried out has a great influence upon the flora which will predominate, e.g., a few udder staphylococci originally present may easily predominate at 37° C., but may be largely suppressed at 18° C. by other types which will then determine the keeping quality of milk. The modifications of the methylene blue test proposed by Wilson, Skar, Thornton and others, may well be used to replace the plate count for certain purposes. This test has the merits of cheapness and simplicity and appears to be much more reproducible than the plate count. For some purposes it is undoubtedly very valuable, but the limits of its usefulness on a large scale in different areas are yet to be determined. For some purposes the modified methylene blue test does not give the information supplied by the plate or the Breed count, but if it is shown that reduction times can be adjusted to give real information as to the history and condition of the milk, it will have widespread application.

Uniformity of treatment as regards details of technique are essential, whatever method is used, if satisfactory comparisons of any kind are to be made between one milk and another.

The presence of heat-resistant organisms in pasteurized milk has recently received attention in this country and in America, and a clear relationship between their numbers and conditions of production has been established in many cases.

Thermophilic organisms, which appear to be without public health significance, are nevertheless an embarrassment to pasteurization. In determining their numbers, the author and his colleagues have encountered difficulties which appear to arise from the uncontrollable effect of the diluent and the interaction of types in the different dilutions (already noted by others).

Some thermophils appear to be very easily killed when cultures are exposed to light in the visible spectrum.

***Prof. C. Gorini** (Milan) : *Types physiologiques des bactéries du lait.*

On trouve trois types physiologiques de bactéries dans le lait : les acidoprotéolytes, les thermophiles et les thermorésistants.

Acidoprotéolytes. Ces bactéries sont capables de peptoniser en milieu acide et de produire non seulement des protéinases mais même des peptidases avec pH optimum en zone acide. En les étudiant il a été possible aussi d'éclaircir plusieurs phénomènes et problèmes de laiterie (coagulation prématurée du lait cru et pasteurisé, maturation du fromage, etc).

Thermophiles et Thermorésistants. La conservation du lait pasteurisé dépend de la qualité de ces deux types. Les plus redoutables sont les sporogènes parce qu'ils sont les plus résistants et ils élaborent des produits dégoûtants, parfois irritants et même toxiques ; heureusement, étant exogènes, ils sont facilement évitables par les mesures hygiéniques. Les nonsporogènes sont inévitables parce qu'une partie est endogène, mais ils sont inoffensifs et antagonistes des sporogènes. Pour le contrôle et la réussite de la pasteurisation, les propriétés physiologiques importent plus que le nombre des bactéries, comme ont observé aussi Anderson et Meanwell, et Wilson.

***Dr. W. Dorner** (Liebefeld bei Bern) : *Significance of Number and Types of Bacteria in Milk for Making Swiss Cheese.*

The addition of pure cultures of appropriate enzyme-bearing micro-organisms to very clean, or to pasteurized milk should offer a method of standardizing the ripening of Swiss cheese. Unfortunately, maturing processes are not scientifically understood and it is not known which bacteria are essential for this ripening. The numbers and vitality of the bacteria also play an important rôle, the growth of necessary bacteria depending upon the composition of the milk and upon its acidity. The acidity in turn depends upon the degree of growth of certain streptococci. Another drawback

to the artificial seeding of clean milk is the difficulty of producing such milk cheaply.

Some of the common contaminants of milk are noted below, and their significance in cheese-making is discussed.

(*a*) *Udder bacteria.* Tubercle bacilli do not interfere with cheese-making, and they are destroyed, probably by the combined action of heat and acidity. Mastitis streptococci do not affect manufacture, but mastitis milk is unsuitable for cheesemaking owing to chemical changes in composition. The rôle played by the other micrococci is doubtful; according to Gorini they can influence ripening. *Brucella abortus* is of no importance and is rapidly destroyed. *Escherichia coli* (*Bacterium coli*) and *Aerobacter aerogenes* (*Bact. aerogenes*) mastitis milk is unsuitable for cheese production owing to gas formation.

(*b*) *Bacteria from cow fæces and feed.* The importance of *Escherichia coli* has been discussed. *Strep. fæcalis* is relatively unimportant. The propioni-bacteria, if too numerous, cause excessive gas formation. *Bacillus amylobacter*, originating from silage, causes blowing in cheese even if present in only small numbers. Clostridia are in themselves harmless unless conditions in the ripening cheese are suitable for their growth, in which case they produce an evil odour.

(*c*) *Bacteria from containers and environment.* Members of the alcaligenes group (*Bact. alkaligenes*) appear harmless. *Strep. lactis* is harmful if too numerous or if allowed to survive at relatively low temperatures, but dies rapidly in properly maturing cheese. Certain micrococci which produce rennet interfere with cheese production, if too numerous. *Strep. thermophilus* and Lactobacilli are found especially if whey is returned to the farms in the milk cans. These two organisms are essential for cheese ripening, but the success of their action depends upon the numbers of each strain which are present. Their presence in quantities greater or less than the optimum amounts renders the ripening process less efficient. Unfortunately, no suitable way of estimating the optimum amount has been devised.

***Mme. Irène Lipska** (Warsaw): *Les colibacilles et les bactériophages du lait de consommation à Varsovie.*

Après avoir étudié dans mes travaux ultérieurs les propriétés biochimiques des colibacilles du lait du district de Skiernieroice (près de Varsovie), ainsi que l'activité des filtrats obtenus de ce lait vis-à-vis des bactéries du groupe coli-paratyphique, j'ai examiné l'influence de la température de l'air et de la nourriture des vaches ainsi que l'effet du transport du lait de la campagne à la ville sur les espèces de colibacilles et sur les races de bactériophages actifs pour le groupe coli-paratyphique.

L'analyse du lait a été faite au mois de mars 1935 (la température minima-9° C. et maxima +3° C.), en ce têmps les vaches sont mal

nourries et ont peu de litière, et au mois d'août de la même année (la température moyenne $\pm$ 20° C.), les vaches se trouvaient aux pâturages et les conditions de la production du lait étaient plus hygiéniques. Cette étude du lait mélangé pris dans les divers lieux de la capitale m'a permis de tirer les conclusions suivantes :

(i) les colibacilles du lait d'hiver ainsi que ceux du lait d'été sont caractérisés par leurs propriétés biochimiques intermédiaires entre les espèces *Bact. coli commune* et *Bact. lactis aerogenes* ;

(ii) les filtrats du lait d'été sont plus actifs que ceux du lait d'hiver vis-à-vis des colibacilles isolés du lait, ceux des déjections animales et humaines et ceux des urines des malades atteints des affections rénales ;

(iii) on observe une analogie dans l'activité des filtrats du lait pour les colibacilles et pour les bacilles typhiques et paratyphiques ;

(iv) la plus grande étendue de l'activité des filtrats du lait d'été embrassait les seize souches sur les vingt-trois bacilles-tests ;

(v) des colibacilles ceux des déjections animales et humaines étaient les plus souvent attaqués, ceux du lait étaient plus résistants, et les plus réfractaires étaient les colibacilles pathogènes isolés des urines des malades.

Vu que la consommation du lait caillé cru est en Pologne une habitude populaire, il serait intéressant de constater si les bactériophages actifs pour le groupe coli-paratyphique gradent leur activité dans ce lait et si, par conséquent, le bon effet curatif du lait caillé peut être attribué non uniquement à l'action de l'acidité du lait, mais aussi à celle des bactériophages.

Un autre point intéressera surtout les pays où a lieu la consommation du lait pur cru (clean milk), c'est de constater si le lait pur est en même temps plus pauvre en bactériophages actifs pour les bacilles du groupe coli-paratyphique et si ces bactériophages sont moins actifs que ceux du lait plus riche en bactéries.

Dr. J. F. Malcolm (Glasgow).

The plate count and coliform tests have proved of great use for advisory purposes in Scotland. The accuracy of the plate count can be greatly increased by the use of milk agar, or tryptone milk agar, instead of ordinary agar ; also by using three or four plates for each dilution instead of one to overcome the effects of uneven distribution of the organisms. As regards the reductase test, we found that in an experiment involving the testing of 10,000 milk samples :

(1) A considerable proportion, 7 or 8 per cent., of samples with low counts fail to pass the reductase test standard of 4½ hours in summer and 5½ hours in winter. (2) A very high proportion of heavily contaminated samples, all with plate counts of over 500,000 per ml., pass the reductase test—in summer about 11 per

cent., in winter from 23 to over 86 per cent., the proportion in winter varying according to atmospheric temperature. Thus in cold winter weather in Scotland comparatively few samples fail to pass the test, irrespective of the extent of contamination. (3) Of 149 samples infected with mastitis, only two samples failed to pass the reductase test, the majority taking 8 hours or more to reduce the dye.

With regard to the reproducibility of results, care should be taken in assessing the value of the test by this method, as erroneous results may be reproduced. Thus in Scotland in winter the results should be highly reproducible, as most of the samples have long reduction periods. The same remarks may apply also to mastitis infected milks.

Prof. W. Riddet (New Zealand).

Milk delivered to cheese factories in New Zealand is compulsorily paid for according to its grade. Milk grading is therefore carried out daily, and a test needs to be used which is reasonably, but not academically, accurate and which can be rapidly carried out with comparatively unskilled labour. Ideally, such a test should take account of both numbers and types of organisms. Unfortunately, too little is known about the specific effects of contaminating organisms in Cheddar cheese to define precisely the test or tests that should be used. Consequently, milk is graded according to (*a*) the type of curd it produces when submitted to a modified curd test, and (*b*) the approximate number of organisms as determined by the Methylene Blue test or Breed's direct count. The introduction of these tests has improved the quality of milk delivered to cheese factories, but difficulties in cheese manufacture still exist because of the importance of starters and the difficulties of preserving their vitality. This may or may not be attributable to the milk flora. The Breed test has been applied with success to three-day composite samples. It has proved very valuable, especially in detecting mastitis and sources of contamination, but its use is limited by the strain on the eyes of observers employed daily on the work. There is need for some method of avoiding this—binocular eyepieces are useful but not entirely satisfactory. Good results are achieved by using the Methylene Blue test for grading purposes, and supplementing it with examinations of smears for the detection of mastitis. Milk grading for cheese-making needs to direct the farmer's attention to producing milk uniform in quality from day to day rather than to define the type of cheese a milk will make.

Dr. K. J. Demeter (München).

Man muss versuchen soweit zu kommen, dass die zur offiziellen Milchkontrolle angewendeten Methoden einheitlich sind, speziell die Plattenmethode. Wir in Deutschland halten daran fest, dass

die Bebrütung der Platten 2 Tage bei 30° C. vorgenommen wird. Das ist einigermassen konform dem neuen amerikanischen Vorschlag von 32° C. Die Begründung, dass die Temperatur von 37° C. deshalb beizubehalten ist, weil die pathogenen bzw. " potentiell " pathogenen Keime bei dieser am besten gedeihen, ist nicht stichaltig, denn die Plattenmethode ist in der offiziell vorgeschriebenen Weise absolut ungeeignet, pathogene Keime auswachsen bzw. als solche erkennen zu lassen, während die " potentiell " pathogenen auch noch sehr gut bei 30 and 32° C. gedeihen. Den Vorschlag von Prof. Breed, an die zweitägige Bebrütung bei 30° einen Tag bei 45° anzuschliessen, werden wir prüfen. Bezüglich der zu verwendenden Agarsorte besteht kein Zweifel, dass Agar mit Kohlehydrat und Milchzusatz bei Prüfung von pasteurisierter Milch dem Standard-Agar unbedingt überlegen ist. Mit Trypton-Agar werden auch bei Rohmilch wesentlich höhere Keimzahlen erhalten. Nur den allgemeinen Keimgehalt festzustellen, ist bei Qualitätsmilchen ein ungenügendes Verfahren, es müssen auch die *Coli-Aerogenes* Keime bestimmt werden (Kindermilch und pasteurisierte Milch !). Welches Verfahren, Plattenmethode oder Gasbildungsnachweis in Elektivnährboden, wäre noch zu entscheiden. An der Methylenblauprobe sind wir in Deutschland deswegen sehr interessiert, weil sie die einzige Probe ist, die regelmässig in der Praxis durchgeführt wird, und Z. Teil auch als Grundlage für die Qualitätsbezahlung dient. Die von G. S. Wilson vorgeschlagene Verbesserung, die Röhren alle ½ Stunde umzustürtzen, werden wir prüfen. Die dadurch verursachte gleichmässigere Verteilung der Bakterien gestattet auch die sonst schwierige Beurteilung von keimarmer Milch.

Prof. S. Orla-Jensen (Copenhagen).

It has been a great pleasure for me to learn about the effort to establish internationally adopted uniform methods of grading milk. Denmark is in the process of working out its standard methods and we hope that co-operation with England, Germany and the United States of America will be possible, so that the results of grading milk can be expressed in a comparable manner. In our laboratory we have for years used nothing but a casein digest medium for the estimation of the numbers of bacteria. So-called sugar-free media may sometimes give counts as high as sugar media, because a small quantity of sugar may have been added unintentionally in the process of cleaning with white of egg. The methylene blue test is particularly useful in cases where the milk is infected with members of the coli-aerogenes group, as these organisms are especially active in the reductase test and also very undesirable in the dairy.

Mr. L. J. Meanwell (London).

In the opening remarks of Drs. Breed and Mattick a distinction was drawn between skilled and unskilled laboratory workers.

Surely it is not so much ease in the actual making of a test that matters, as ease in the interpretation of results. Hence, workers trained in bacteriology and dairy practice should be engaged in milk control work.

When comparing the methylene blue and the plate count tests, it should be remembered that the methylene blue test detects highly contaminated milks, but that to trace the source of contamination the plating method is necessary. In conclusion, the speaker asserted that in commercial practice the methylene blue reductase test at 63° C. has been found invaluable.

Dr. K. J. Demeter (München).

Ich möchte Herrn Dr. Meanwell fragen, wie er sich auf Grund seiner Beobachtungen zu folgender Tatsache stellt. Die von uns zur Resistenzprüfung verwendeten *Coli*-Stämme werden zunächst auf Agar 14 Stunden bei 37° C., dann 24 Stunden bei 20° C. bebrütet, um dann 2 Stunden vor dem Versuch in die Milch abgeschaümt zu werden. Unsere Stämme sind sehr hitzeresistent. Würden Sie diese Kulturen nun als im Stadium des physiologischen Jugendzustandes befindlich betrachten oder nicht?

Zu Dr. Meanwells Vorfrage und Dr. Walkers Bemerkungen können wir in Übereinstimmung mit Hennebergs Ergebnissen, ebenfalls bestätigen, dass in der Natur wirklich hitzeresistente *Coli*-Stämme äusserst selten sind. Alle hochresistenten Stämme, die wir besitzen, haben sich diese hohe Resistenz erst im Laufe der künstlichen Kultur erworben.

Dr. A. Cunningham (Edinburgh).

Comparison of the Burri Smear method and the plate method showed that the former was much less accurate than the latter. The lesser accuracy of the Burri method has been attributed to fallacies of loop measurements and to the inferiority of conditions on the surface to those in the depths of a medium.

American Standard Agar, Milk agar and Wilson's agar have been compared for the examination of raw milk. In most samples little difference has been detected. In a certain number the two latter media were found to be markedly superior to the first. The presence of mastitis streptococci in such samples was suspected but confirmatory evidence could not be obtained.

The speaker considered that at present there was no justification for substituting the methylene blue test for the plate count in the examination of high-grade milk.

Prof. S. Orla-Jensen (Copenhagen).

I should like to emphasize that the methylene blue reductase test is most successfully used in connection with the fermentation test. The former is principally a quantitative test, bearing on the

ceeping quality of the sample, and is related to proper cooling. The fermentation test, however, is a qualitative test concerned vith the general cleanliness under which the milk has been proluced. I admit that different tests may be used according to the bject of examination, but I do contend that the reductaseermentation test is a very easy and simple test for use in dairies nd particularly in cheese factories. In Denmark it would be quite mpracticable to replace it by any of the other tests.

Dr. P. Arne Hansen (Copenhagen).

The use of an incubator working near 37° C. seems disadvantageus in practical milk testing, as compared with one working at 32° C. This is brought out by the graph which Dr. Breed has hown. If the temperature of the incubator varies above 37° C., t may come very close to the limit of growth for some of the ordinary acteria occurring in raw milk. Most strains of *Streptococcus cremoris* lo not grow at 39° C.

Dr. K. J. Demeter (München).

Wenn tätsachlich echte Bakterizidie vorliegen würde, müssten ich meiner Ansicht nach im Verlauf der Lagerung der Milch rgendwelche Unterschiede in der Resistenzfähigkeit zeigen, abnehnend mit der Dauer der Aufbewahrung. Bei den Versuchen in Kiel wurde etwas ähnliches beobachtet, bei uns nicht. Es gelang ins auch bei wiederholten Versuchen nicht, bezüglich des llgemeinen Keimrückganges der eingeimpften *Coli*-Keime 2–8 tunden nach Beimpfung einen Unterschied zwischen roher und rhitzter Milch zu finden.

Mr. Ben Davies (London).

In discussing the bacteriological testing of milk, it is to be hoped hat workers will not expose themselves to the accusation that is ometimes advanced against the medical profession, namely, that hey are more interested in the study of disease than in promoting ealth. The greatest service that can be rendered by bacteriogists is to insist at every season that without the resources of good uildings, elaborate equipment, and ultra-efficient refrigerators, : is possible for every producer, by regard for the principles of rdinary decency and common cleanliness, together with the terilization of utensils and appliances, to produce milk of the ighest bacteriological character. In the case of such decently roduced milk the so-called bacteriological quality of milk limits ny material increase in bacterial counts for from 12 to 20 hours nder conditions of ordinary room temperatures. There should be o toleration for supplies which, in consequence of carelessness and egligence, are inferior in this respect. With such compulsorily lean milk laboratory control is simple and reliable.

DISCUSSION *on* FACTORS DETERMINING THE BEHAVIOUR OF MICRO-ORGANISMS IN MILK AND MILK PRODUCTS

Chairman : Prof. S. Orla-Jensen.

Mr. L. J. Meanwell (London), **Opener** : *The Influence of Environment on Thermal Death of Bacteria.*

From a purely commercial point of view the question of resistance of bacteria to environmental changes, particularly temperature changes, plays a most important part in the technical processes of the dairy trade. A consideration of those factors that have been shown to influence the resistance of organisms to heat may well be placed at the opening of a series of papers dealing with the behaviour of micro-organisms in milk and milk products.

One important factor affecting the resistance of micro-organisms to the application of heat has been shown by Sherman and Albus (1924) to be the physiological age of the organisms at the time of heating.

"*Physiological Youth*" at the optimum growth temperature of *Bacterium coli* has been found to result in greatly reduced resistance of the organism to heat and other environmental changes. This reduced resistance is accompanied by a greatly increased rate of metabolic activity not entirely explained by increase in cell size. In practice it has been found that under certain conditions it is possible to treat milk by heat at a time when the majority of organisms present are in their least resistant stage of growth, and by this means certain outbreaks of ropy milk have been successfully combated.

It has been suggested that "slow growth" should lead to a more perfect adjustment of an organism to its environment, and recent work appears to support this view. The fact that slow growth may lead to an actual *increase* in resistance immediately prior to and following the initiation of growth has recently been shown by Anderson and Meanwell (1936). These workers studied the heat resistance of certain streptococci isolated from pasteurized milk and found that when test organisms were cultured at temperatures below the optimum for growth, there was a progressive increase in heat resistance during the lag and early logarithmic phases. This increase in resistance became more marked the further the temperature of growth was removed from the optimum, but at all test temperatures the resistance showed a steady decline by the time a tenfold multiplication of the unheated organisms had taken place.

The importance of these finding lies in their possible application to everyday dairy practice. Milk produced under certain conditions may contain a bacterial flora that will increase in resistance on storage. For example, it has been found in some districts that the storage of raw milk at temperatures permitting slow multiplication

does result in the development of an exceedingly resistant flora—such conditions are quite common in those districts where milk is delivered to the dairy twice daily and the practice of farm cooling is replaced by refrigeration at the milk receiving depot.

Anderson and Meanwell also found that if refrigeration was applied for eighteen hours upon an organism in the early logarithmic growth phase the heat resistance of the culture on subsequent pasteurization was enormously increased, but refrigeration applied to organisms in the more advanced logarithmic phase of growth had no measurable effect.

The influence of acidity or *p*H on heat resistance has been found to be of great importance in the canning industry, and more recently it has been demonstrated that an increase in the concentration of hydrogen ions in milk may result in a marked decrease in the heat resistance of any organisms present. This observation is of great commercial significance, as its application to practical conditions will condemn the not uncommon practice of mixing sweet with slightly sour milk, in order to prevent the loss of the latter. By mixing in this way it is quite possible that the resistance of the large number of organisms present in the slightly acid milk may be considerably increased.

Fay (1934) and Baumgartner & Wallace (1934–36) have clearly demonstrated the protective action of sugar in the processing of canned fruits, condensed milk, etc. These workers (Baumgartner & Wallace) found that the resistance of micro-organisms in sugar solution increased not only to heat but also against the action of protein coagulating disinfectants. When an oxidizing germicide such as hydrogen peroxide was used, sugar afforded no protection at all.

***Prof. C. Gorini** (Milan) : *Température, air et qualité du lait.*

Parmi les facteurs du comportement des bactéries dans le lait et les produits laitiers, les quatre suivants ont une importance capitale : température, air, qualité du lait, association symbiotique.

Température et air. Les températures basses et l'aérobiose favorisent la caséolyse, les températures hautes et l'anaérobiose favorisent la saccharolyse.

Qualité du lait. La manière de stérilisation et la nature du lait sont importantes. Certaines bactéries et certaines fonctions sont favorisées dans le lait autoclavé, d'autres dans le lait tyndalisé.

Il y a des laits qui, quoique normaux aux examens ordinaires, sont modifiés dans leur propriétés biochimiques de sorte qu'ils sont défavorables à la culture des ferments lactiques et acidoprotéolytes et, par conséquence, à la fabrication du fromage. Cette propriété persiste dans le lait stérilisé ; vraisemblablement elle consiste en une carence, pouvant être supprimée par des stimulants (peptone, sang, vitamines). Cette sorte de lait a été observée par plusieurs rechercheurs, mais on ne connaît pas les causes de sa production.

Mes recherches démontrent que l'introduction dans la ration alimentaire des fourrages inférieurs fait paraître ce lait ; l'addition d'herbe verte empêche l'action dangereuse des dits fourrages.

*Dr. **H. R. Whitehead** (Palmerston North, New Zealand): *Lactic Acid Bacteria in the Manufacture of Dairy Products.*

Control of the growth of bacteria of the lactic acid group is essential in the manufacture of dairy products. The communication discusses recent work on the influence of the following factors:

(1) Composition of the medium in which the bacteria are required to grow, in respect of,

- (*a*) natural variations in milk composition. Thus, the proportion of lactose is important, whilst certain batches of milk do not support the growth of streptococci so well as others, although there is no evident difference in their gross chemical composition.
- (*b*) influence of various additions to and treatments of the medium. The addition of yeast, tomato extract or hydrolysed protein improve the medium, which indicates that activating substances present in small amounts, and occasionally lacking in certain batches of milk, play a large part.

(2) Temperature. Whereas many lactic streptococci are indistinguishable at 30° C., some fail to grow, or otherwise develop involution forms at higher temperatures. In cheese manufacture, for instance, where acid development is required to proceed at higher temperatures than 30° C., strains selected on a basis of their higher optimum temperature are much more satisfactory for use.

(3) Inhibition or stimulation by products of bacterial growth. Since all milk contains adventitious micro-organisms, the effect of these organisms or their products may have an important bearing on manufacturing processes. Thus, certain strains of lactic streptococci form a satisfactory symbiotic mixture with certain strains of Betacocci, resulting in adequate growth of the latter with the production of diacetyl flavouring substances. Again, it is extremely difficult to isolate lacto-bacilli from fresh milk, but when such milk has been soured by lactic streptococci to a sufficient degree of acidity, lacto-bacilli appear as the predominant flora. As an example of antibiosis, certain lactic streptococci develop a product strongly and specifically inhibitory for normal lactic streptococci. Milk, once contaminated with this product, remains a poor medium for the normal streptococci, even after pasteurization.

(4) Occurrence of bacteriophage in cultures of lactic acid organisms. Recent work has shown that, so far as lactic streptococci are concerned, the phages which attack these organisms are probably the most potent influence affecting their growth and activity in milk. The origin of the phages, which in this case seem to be strain-

specific, is unknown. The presence of phages in cultures of lactic streptococci most probably explains some of the sudden variations in growth which have been observed in the past.

Dr. J. G. Davis (Shinfield, Reading) : *The Respiratory and Fermentative Mechanisms of the Lactic Acid Bacteria.*

The rate of growth of bacteria in milk and other media is primarily a question of their ability to obtain available energy. Of the two main sources of energy—oxidative and fermentative reactions—the latter release only about 5 per cent. the energy obtainable by the former. The lactic acid bacteria, however, utilize fermentation reactions predominantly, if not entirely, for their growth purposes. Their behaviour towards oxygen depends on the vigour of the strain and the types of respiratory enzymes in the cell. Controlled experiments in dextrose broth under definite oxygen tensions show that aeration of a vigorous culture of *Str. lactis* results in a shorter lag period. There is thus some evidence that oxygen favours the initiation of growth. The sequence of aeration conditions in the souring of milk is probably eminently suitable for the lactic acid bacteria.

We may therefore ascribe the predominance of lactic acid bacteria in milk, cheese, etc., to (1) their ability to utilize casein, (8) their ability to ferment lactose, and (3) to suitable aeration conditions in milk.

Old cultures of *L. delbrueckii* (e.g., 3 days at 37°) may have entirely lost their respiratory power, but will retain their glycolytic or acidogenic power undiminished. Washing of the cells also greatly reduces the respiratory activity, which appears to be activated by substances attached to the surface of the cell or perhaps easily diffusible. The acidogenic power is not greatly reduced by washing. Weakened cultures may develop an " oxygen sensitivity," due to a disturbance of equilibrium between (1) the rate of diffusion of atmospheric oxygen into the medium and the cell, and (2) the removal of this oxygen by the intermediate products of fermentation. In weakened strains, factor (2) falls behind (1).

The lactic acid bacteria fall into broad groups when classified according to their respiratory enzymes—these confirm, in general, the system of Orla-Jensen. Cocci and rod forms may be placed in physiologically equivalent groups, according to their possession of any or none of the following respiratory systems: Warburg " flavine," hæmochromogen, and catalase.

Dr. K. J. Demeter und **Dr. L. Eisenreich** (München) : *Erfahrungen bei der bakteriologischen Prüfung von neuzeitlichen Milcherhitzungsapparaten* (*Verwendung hitzeresistenter Coli-Stämme*).

In den deutschen Vorschriften für Erhitzerprüfung ist vorgesehen, dass in der Regel durch Kurzzeiterhitzer bei 70° C. solche

Coli-Stämme abgetötet werden müssen, die eine Resistenz von mehr als 25 Minuten und weniger als 30 Minuten bei 62° C. besitzen. Bei den praktischen Prüfungen ergaben sich diesbezüglich grosse Schwierigkeiten, deren Aufklärung dringend geboten schien.

(1) Feststellung von Faktoren, die die Hitzeresistenz der Coli Kulturen herabsetzen.

Da die Art der vorhergegangenen Bebrütung keinen eindeutigen Einfluss erkennen liess, interessierte uns in erster Linie der evtl. Einfluss des Substrats, in dem die Resistenzprüfung vorgenommen wurde. Bei *Rohmilchen* verschiedener Herkunft ergaben sich keine wesentlichen Unterschiede, bei Milchen *derselben* Herkunft änderte sich jedoch die Resistenz der eingeimpften Coli-Keime oft beträchtlich. Obgleich die Beständigkeit bezüglich Hitzeresistenzerhaltung in den Sommermonaten etwas geringer zu sein schien, liess sich irgend ein Futtereinfluss nicht erkennen.

Im weiteren ergaben sich ganz auffallende Unterschiede in der Resistenz *derselben* Coli-Stämme, wenn diese in der *Rohmilch* und gleichseitig auch in *sterilisierter Magermilch* im Ampullenversuch nachgeprüft wurde. In der sterilisierten Milch war die Resistenz in der Regel wesentlich höher als in der unerhitzten Vergleichsmilch. Diese Beobachtung war die Veranlassung zu den folgenden Untersuchungen.

(2) Untersuchungen über die Art des in Rohmilch vorhandenen wirksamen Hemmungsfaktors.

(*a*) Der Einfluss vorhergehender Erhitzung der Milch auf die Hitze-Resistenz der Coli-Stämme.

Mit Ausnahme von 3 Fällen, in denen gar kein Unterschied beobachtet wurde, betrug die Resistenzminderung in Rohmilch 10–60 prozent des Wertes in der sterilen Magermilch. Diese resistenzvermindernde Wirkung der Rohmilch konnte durch ein wenige Sekunden dauerndes Erhitzen derselben auf 80° C. aufgehoben werden. Beim Erhitzen auf 75° waren im allgemeinen schon 3 Minuten erforderlich, beim Erhitzen der Milch auf 50–70° liessen sich in dieser Richtung keine Gesetzmässigkeiten mehr aufstellen. Es erhebt sich die Frage, ob das wirksame Agens nicht aus 2 *Faktoren* besteht, von denen der eine bei tieferen Temperaturen, der andere erst zwischen 75 und 80° C. ausgeschaltet wird.

(*b*) Beziehung zwischen Zeitdauer der Exposition in Rohmilch und Hitzeresistenzabfall.

Rohe Milch wurde mit einer Abschwemmung resistenter Colistämme beimpft und deren Resistenz in bestimmten Zeitabständen nachgeprüft. Da sich die Resistenz der Stämme hierbei nicht eindeutig nach irgend einer Richtung veränderte, kann von einem bestimmten Einfluss der Expositionszeit auf die Resistenzfähigkeit nicht gesprochen werden. Eine Abwandlung dieser Versuche insofern, als die rohe Milch erst unbeimpft im Kühlraum aufbewahrt und dann erst nach 24 oder 48 Std. mit resistenten Stämmen

geimpft wurde, ergab ähnliche Resultate. Daraus lässt sich schliessen, dass das wirksame Agens während der Aufbewahrung in seiner Wirksamkeit nicht wesentlich beeinträchtigt wurde, wie dies bei der eigentlichen Bakterizidie zu erwarten gewesen wäre. Bei einem Rückgang hätte allmählich der Resistenzwert erreicht werden müssen, wie er in vorerhitzter Milch vorhanden war. Dies war aber nicht der Fall, da die Schwächung der Resistenz von Anfang an im Prinzip aufrecht erhalten blieb.

(*c*) Versuche zur Erfassung des wirksamen Prinzips.

Es wurden bei der Lab-Molke analoge Zusammenhänge wie bei der Milch gefunden, aber in verstärktem Ausmasse. Wurde die rohe Molke mittels Aether extrahiert und der Aether im Vakuum bei sehr niedriger Temperatur wieder entfernt, so ging die Resistenz der Coli-Stämme in solcher Molke auf alle Fälle sehr stark zurück ; aber auch in solcher nach dem Extrahieren auf 80° erhitzten Molke war die sonst beobachtete Verbesserung der Resistenz mit einer einzigen Ausnahme nicht mehr feststellbar. Entweder wirkten die zurückbleibenden Aetherspuren direkt auf die Bact.-Coli ein, oder es sind die wirksamen Substanzen durch den Aether nicht entfernt worden bezw. es hat durch ihn irgend eine Stabilisierung der betreffenden Substanz gegenüber Hitzewirkung stattgefunden. Durch Behandlung der Molke mit Tierkohle ergab sich im allgemeinen sowohl bei der rohen Molke als auch bei der nach Kohlebehandlung erhitzten Molke eine wesentliche Steigerung der Hitzeresistenz der eingeimpften Coli-Bakterien. Anscheinend wurde durch die Tierkohle das hemmende Agens nicht ganz adsorbiert, da die nachträgliche Erhitzung der von der Tierkohle abzentrifugierten Molke auf 80° C. noch eine weitere Steigerung der Hitzeresistenz zur Folge hatte. Auch diese Beobachtung deutet darauf hin, dass vielleicht 2 Faktoren vorhanden sind, von denen der eine durch Kohle adsorbierbar ist, der andere nicht. Weitere, noch nicht abgeschlossene Versuche lassen Beziehungen zu oxydativen Prozessen erkennen.

(3) Beziehungen zwischen der Resistenz der Coli-Stämme in roher und erhitzter Milch und die Pasteurisierwirkung bei der Kurzzeiterhitzung.

Im allgemeinen war die Pasteurisierwirkung umso geringer, je höher die Hitzeresistenz der Bakterien in der *Rohmilch* war. Es sind jedoch auch häufig Fälle vorgekommen, wo bei hoher Resistenz eine viel bessere Wirkung der Kurzzeiterhitzung erzielt wurde, als bei niedrigerer Resistenz. Die kurze Dauer der Einwirkung höherer Hitzegrade in den Kurzzeiterhitzern lässt der Milch nach unseren Versuchen diesbezüglich den *Charakter einer Rohmilch unter Erhaltung der in dieser ursprünglich enthaltenen, die Hitzeresistenz herabsetzenden Substanzen.* Wenn z.B. folgender Fall vorliegt :

Resistenz bei 62° C. in vorher auf 80° erhitzter Milch 40 Min.
Resistenz bei 62° C. in roher Milch 20 Min.,

dann wird die Resistenz durch die Einwirkung nicht der Hitze, sondern der die Resistenz hemmenden Substanzen um 50 prozent geschädigt. Wäre keine Beeinträchtigung der Hitzeresistenz vorhanden gewesen, hätten die Keime die 40 Min. lange Erhitzung auch in der Rohmilch aushalten müssen. Infolgedessen hat der betreffende Apparat bei Abtötung dieser Keime aus eigener Kraft eigentlich nur Stämme abgetötet, die für ihn unter diesen Umständen eine Resistenz von nur 20 Min. hatten. Hierbei muss die Voraussetzung gelten, dass die geringe Dauer der Hitzeeinwirkung im Kurzzeiterhitzer schon genügt, den Hemmungsstoff zur Wirkung kommen zu lassen, wie dies unter allen Umständen beim Ampullenversuch oder der Dauererhitzung während 30 Min. der Fall ist. Dann ist auch die Bezugnahme der Resistenz auf die Rohmilchverhältnisse gerechtfertigt. Ist das nicht der Fall, dann ist der Kurzzeiterhitzer unter verschärften Bedingungen geprüft, weil er ohne Mitwirkung des hemmenden Agens tatsächlich Stämme von 40 Min. Resistenz und nicht von 20 Min. (+ Agens) abgetötet hat. Eine weiterhin zu klärende Frage ist die, ob das resistenzvermindernde Agens nicht durch die Hitze erst richtig aktiviert wird.

Die Folgerung aus unseren Ergebnissen und aus ähnlichen, unabhängig von uns an der Kieler Anstalt durch *Lemcke* durchgeführten Untersuchungen ist jedenfalls die, dass wegen der Kompliziertheit der Vorgänge eine Begutachtung von Kurzzeiterhitzern auf der bisherigen Grundlage der Coliversuche nicht mehr befürwortet werden kann.

Dr. M. Grimes (Cork, I.F.S.): *The Influence of Temperature on the Estimation of Bacteria in Ice-Cream.*

In co-operation with Mr. A. J. Hennerty a study has been made of the heat tolerance in ice-cream mix of *Escherichia coli, E. formica, E. paragrünthali* and *E. pseudocoloides* isolated from ice-cream. The total count of the cultures heated at 145° F. for thirty minutes compared with the total count of the same cultures before pasteurization showed that the pasteurization temperature used killed 99·9 to 100 per cent. of the bacteria originally present. Our results confirm those previously reported that certain strains of the Escherichia group may survive heating in ice-cream mix at 145° F. for thirty minutes. It was noted that the greater the original inoculation, the more likely that a number of cells survived the temperature of pasteurization. The motility of bacteria was found to be influenced by both temperature and the presence or absence of carbohydrate in nutrient broth. In our work with the Aerobacter species[1] that produce acid and gas in nutrient lactose broth at 21° C., it was found that motility was most noticeable at 21° C. variable at 30° C. and hard to determine at 37° C. The most actively motile bacteria were the smaller ones—0·75μ to 0·9μ by

[1] *Sc. Proc. Roy. Dub. Soc.*, **1931**, **20** (N.S.) 89.

1·0μ to 1·25μ, and as the size increased the bacteria tended to become sluggish. When grown at 37° C. most of the bacteria tended to be 1·0μ by 2·0μ. When these Aerobacter species were grown in Dunham's solution (incubation temperature 21° C.) motility was evident, but when grown in nutrient lactose broth, motility was not evident, so that a bacterium should not be classed as non-motile without checking motility after growth in different liquid media and at different temperatures.

In co-operation with Mr. J. L. Shimwell[2] a study was made of *Flavobacterium proteus*. This was found to give best growth in nutrient broth in which the percentage of peptone and meat extract were doubled. It was also found that in wort, the adjustment of the media to *p*H 5·0 gave better growth.

The question of adaptation of bacteria to their environment is very interesting. If milk is held for a considerable period at low temperatures, types will establish themselves after some days that grow readily at these low temperatures, and as these types are slow reducers of methylene-blue, the methylene-blue test is not a reliable guide to the bacterial count of such milk.[3]

A series of 79 experiments, using duplicate plates and following standard methods, to compare the total bacterial counts of different samples of ice cream grown on nutrient lactose agar and incubated at 21° C. and at 30° C. respectively for five days, and at 37° C. for two days was made. The total counts varied (plate count at 21° C.) from less than 100,000 to over 100,000,000 per grm. ice-cream. It was found that the average plate count at 30° C. was 86 per cent. of that at 21° C., with a maximum percentage of 115 and a minimum percentage of 47. The average plate count at 37° C. was 35 per cent. of that at 21° C., with a maximum percentage of 73 and a minimum of 3. No matter what incubation temperatures are used, at times there will be freak results, due to the species of bacteria chiefly present in the dairy product under examination. It has been noticed during the examination of different ice-creams that while plates usually required five days incubation at 21° C. or at 30° C. to reach maximum colony growth, at times plates were ready to count after two days' incubation.

Dr. P. Kouchakoff (Lausanne): *The Effect of Milk as a Food upon the Leucocyte Formula of Man.*

Milk is our universal food—therefore, the question of how to keep its original properties by preserving it is naturally of great importance. Our problem was to find out whether there was a reaction in our bodies due to the various methods of preserving it.

In a previous report, at the 1st International Congress for Microbiology (1930) and the International Physiological Congress in

[2] *J. Inst. Brew.*, 1936, **42**, 348.
[3] Grimes, M., *Le Lait*, 1929, **9**, 905.

Rome, 1932, we stated that alimentary leucocytosis did not represent a physiological phenomenon.

Virchow's recognition of "alimentary leucocytosis"—which is the name given by him to the increase in the number of leucocytes occurring after each meal—as a physiological phenomenon, represents one of the greatest scientific errors, as the result of which the work of all subsequent investigators was based on false premises. Our own experiments have shown that "alimentary leucocytosis" does not take place after consumption of food in the form in which it was produced in nature, but that leucocytosis occurs after consumption of food altered by high temperatures and by complicated manufacture. The alimentary leucocytosis serves as an indicator of some pathological process taking place in the organism as the result of the introduction of food which is not readily assimilable.

Further experiments on milk have shown that its critical temperature was 88° C. at sea level. (By the term "critical temperature" we mean the highest temperature at which a particular foodstuff can be heated for half an hour, without changing our blood formula, when consumed. If this temperature is exceeded, milk loses its natural properties and causes an increase of leucocytes). On adding to milk certain manufactured products, such as cane sugar, not only is the number of leucocytes increased, but a change takes place in the percentage of the various types of white cells.

Milk containing an abundant bacterial flora, as well as milk from tuberculous animals, has no effect upon the leucocyte formula of man.

If milk has become altered by overheating alone, its original properties can be restored in the following manner: (1) by addition of 5 to 10 per cent. of raw milk, or of any other raw product the critical temperature of which is equal to that of milk or higher; (2) by keeping the boiled milk exposed to air for 24 hours; (3) by addition of saprophytic microbes (sour milk, yoghourt, etc.).

If cane sugar is added to boiled milk, the reaction of the organism to this mixture can be prevented only by adding to it three raw products, provided their critical temperatures are different.

The natural properties of milk cannot be restored by any method, if it has been heated under higher pressure than atmospheric.

Our investigations have led to the following conclusions: (1) Natural milk and all its derivatives (cream, butter, cheese, sour milk and milk dried by low temperature—pure or with admixture of lactose) do not alter the leucocyte formula of man; (2) Boiled and condensed milk, with admixture of cane sugar, or other prepared products, cause a change in the leucocyte formula, thus representing unnatural foodstuffs, assimilable with difficulty; (3) The alteration of the leucocyte formula can serve as a test for the differentiation of natural from adulterated foodstuffs, applicable to the examination of market products; (4) The leucocyte formula provides no clue for

determining whether the milk orginated from healthy or diseased cows ; and (5) The examination of milk from six tuberculous cows in all stages of the disease failed to reveal the presence of tubercle bacilli, either microscopically or by inoculation of guinea-pigs ; therefore, the risk of human infection through consumption of raw milk must be very limited.

It seems to us that it is important whenever we find a new way of preserving milk, to apply the rules which I have given above.

Dr. K. J. Demeter (München).

German legislation has prescribed an official test for all types of pasteurizing apparatus. Part of the test consists of using heat-resistant strains of *Bacterium coli*, which are supposed to resist 62° C. for 25 minutes but are killed by this temperature acting for 30 minutes. They must regularly be destroyed by 70° C. Examining the factors which may influence heat-resistance, the speaker concluded that neither the previous method of cultivation nor the medium influenced the resistance. Considerable difference in the resistance was found between suspensions of the organisms in raw and in sterile skimmed milk. This factor present in raw milk, which diminishes the resistance of coli bacilli, has been studied. Storage of the milk did not affect it. Its action in sterilized milk could be replaced by additions of hydrogen peroxide. The factor could be adsorbed on to kaolin but could not be eluted from this adsorbant. There is evidence of some connection between this factor and the peroxydase content of milk. On account of the irregular occurrence of this factor in milk, the heat-resistance of *Bact. coli* is an unreliable test for pasteurization.

Dr. Z. Herschdörfer (London).

Many children are fed with dry milk or similar preparations. There is much reason for believing that the milk used for such preparations is not of high grade and not of high bacteriological quality. It seems important to investigate whether tubercle bacilli can survive in the preparations, and whether it would not be advisable to include dry milk in the grading system introduced for fresh milk.

Dr. F. C. Minett (London), requested further information from Professor Kouchakoff regarding his conclusion No. 5 (Abstracts of Proceedings, p. 113), in which it was stated that " the examination of milk from tuberculous cows in all stages of the disease failed to reveal the presence of tubercle bacilli, either microscopically or by inoculation of guinea-pigs." The danger to human beings from the consumption of milk containing bovine tuberculosis bacilli was too well known to need elaboration, but it might be unfortunate if a statement such as that made by Professor Kouchakoff was allowed to pass unchallenged.

Dr. P. Kouchakoff (Lausanne).

In reply to the suggestion that the consumption of dried milk by new-born infants tended to increase the incidence of tuberculosis among them, probably owing to the fact that this method of preparation did not kill the tubercle bacilli, Dr. Kouchakoff pointed out that, according to his method, milk should be consumed in its natural state heated to a temperature not exceeding 88° C. for half an hour. Under these conditions the milk retained its original properties (as regards the leucocyte formula) and, moreover, this temperature was high enough to destroy the tubercle bacilli which might be present in the milk.

If dried milk was prepared after a preliminary heating not exceeding 88° C., the risk of infecting the new-born with tuberculosis was reduced to zero. As regards condensed milk to which cane sugar had been added, or which had been heated under pressure above the atmospheric, in Dr. Kouchakoff's opinion, such milk represented merely a substitute. It was common knowledge that alcohol predisposed to infection with tuberculosis, and sugar (as far as the leucocyte formula of man is concerned) belonged to the same group as alcohol in its effects.

In reply to Dr. Minett, Dr. Kouchakoff stated that his object was to determine the effect of milk from tuberculous cows upon the leucocyte formula of human blood. He made experiments with milk from six cows in different stages of tuberculosis—up to the miliary stage—and at the same time examined the milk for the presence of tubercle bacilli. The latter could not be detected either by direct observation or by inoculating guinea-pigs, from which it was concluded that the milk of tuberculous cows did not invariably contain tubercle bacilli.

The possible infection of modern man with tuberculosis from a bovine source was not denied, but it was pointed out that it would be impossible to infect a man in perfect health through the alimentary tract, since normal stomach juice contained from 2 to 2·5 gm. of hydrochloric acid per litre of fluid, which represented an antiseptic solution. The fact that the leucocyte formula of human blood remained unchanged not only after consumption of milk from tuberculous cows, but after consumption of other pathogenic microbes as well, was in itself sufficient proof that this point was not vulnerable in a healthy body.

In the future it might be possible to create healthy human beings by correct nutrition from the first days of life. The criterion of correct nutrition was provided by the leucocyte formula which should be maintained at its normal physiological level. All foodstuffs, such as preserves, etc., should satisfy these reqnirements and should be under State control. As regards milk, there was no necessity to consume it raw, since—as shown above—when heated up to 88° C. its physical properties with regard to the leucocyte formula of the

blood remain unchanged, while this temperature was also sufficient to render milk safe as regards tubercle bacilli.

Mr. L. J. Walker (London), **Prof. S. Orla-Jensen** (Copenhagen), **Mr. L. J. Meanwell** (London) and **Dr. A. T. R. Mattick** (Shinfield, Reading) also took part in the Discussion.

SUBSECTION 2—WATER SEWAGE AND INDUSTRIAL MICROBIOLOGY

DISCUSSION *on* THE MICROBIOLOGY OF WATER SUPPLIES

Chairman : Prof. R. E. Buchanan (Ames, U.S.A.).

Prof. F. E. Fritsch (London), **Opener** : *The Problems presented by the Algal Population.*

The problems presented by the algal population in a body of water largely concern the occasional abnormal development of one form or another. In the main such abnormal development takes place in the phytoplankton and, when carried to an extreme, leads to the formation of water-flowers, for which blue-green algæ are most usually responsible. At the same time excessive multiplication of certain diatoms, while not as disastrous in its general effects, may be the cause of infinite trouble in sources of water supply.

The primary problem involved is that of the origin of the phytoplankton, in the annual development of which one can always distinguish a succession of phases whose dominant forms are at other times of the year only scantily represented or apparently completely absent. The annual recrudescence of the members of a given phase can only take place from two sources, the littoral zone and the open water itself. There is some evidence that certain planktonic diatoms survive in the littoral zone, and that a new phase takes its origin from that source. There is, however, little to indicate that this is universal for planktonic diatoms, and it does not appear to apply to the blue-green algæ. A certain number of the latter produce spores which sink after formation and constitute the means of survival, but for many planktonic blue-green algæ (including some of those producing water-flowers) no such durable structures are known. In these, survival must be due to persisting vegetative individuals, and either so few persist that their presence is overlooked in ordinary plankton observations or the surviving individuals sink to the bottom or into deeper strata of the water.

A large number of the blue-green algæ of the plankton possess gas-vacuoles which render them buoyant, and recent observations have made it probable that these gases result from fermentative activities within the cells under anaerobic conditions. The spores possess few or none of these vacuoles and consequently have not

the buoyant properties of the vegetative cells. In the same way it is possible that other species, which do not produce spores, survive as individuals that contain no gas-vacuoles and consequently sink into deeper water. Recrudescence in either case would then be due to the realization of conditions causing a formation of gas-vacuoles, with a consequent rise to the surface. It is not out of the question that similar phenomena may come into play in other members of the phytoplankton. Physiological changes within the cells inducing alterations in specific gravity may condition at one time the sinking, and at another the rise, of ordinary vegetative individuals.

Other problems centre around the causes of the excessive multiplication which are still altogether obscure. Water-flowers can be formed at all times of the year, although more prevalent in the warmer months; in successive years they may, in the same body of water, be due to different species. The facts indicate that temperature and light-intensity are probably of minor importance, and that the chief factors are to be sought in the chemical constitution of the water. At the present time we have little direct knowledge as to the extent to which active multiplication of a given algal species is dependent upon the presence of certain necessary mineral ingredients in adequate amount and in suitable relative proportions. The extensive experience obtained in laboratory cultures, however, has shown that even slight variations in the amounts of the ingredients supplied may mean all the difference between an impoverished and a healthy growth. Nitrates, phosphates, and iron salts are among the most important, but the hydrogen-ion concentration of the water is no doubt also of considerable significance.

It is becoming increasingly evident that the type of littoral sediments has an important bearing on the kind of algal flora in a piece of water, since the former not only differ widely in chemical and physical characteristics, but also in the flora which they bear and breed. As a possible means of control, too, more attention, will have to be paid to the micro-fauna and especially to those members of the latter, like certain Rotifers and Ephemerid-larvae, which seem to be selective feeders.

Prof. F. Dienert (Paris): *Considérations sur la microbiologie des eaux.*

L'analyse bactériologique des eaux a pour but essentiel de connaître si, au moment de l'analyse, l'eau recevait plus ou moins de germes suspects.

La numération de tous les germes de l'eau, sans distinction de leur nature, apparaît comme une recherche inutile; en tout cas, cette numération totale dépend beaucoup de la nature du milieu sur lequel on la fait—elle est généralement inférieure sur gélose que sur gélatine. Pourtant, les germes qui ne cultivent pas dans nos

milieux ordinaires de culture, les protozoaires par exemple, ne sont pas négligeables ; et il existe des méthodes spéciales, mais en même temps suffisamment pratiques, pour les déceler et les numérer.

La recherche des protozoaires donne d'utiles indications sur la contamination plus ou moins grande des eaux. En outre, certains protozoaires sont des agents actifs d'épuration ou même indispensables, e.g., les colpodes.

On peut obtenir d'utiles renseignements en recherchant la présence du bactériophage dans les eaux, une recherche qui est souvent negligée. L'arrivée de germes pathogènes dans les eaux est accompagnée presque toujours de celle du bactériophage correspondant, principe plus facile à rechercher et à mesurer.

Quant aux germes à rechercher pour caractériser les eaux contaminées, en France on recherche le *Bact. coli*, l'entérocoque, les microbes producteurs d'hydrogène sulfuré et le perfringens. Si l'un de ces germes est rencontré, l'eau est suspecte, mais il faut s'assurer que la méthode de prélèvement de l'eau a été précise. En France, les milieux phéniqués, sont les milieux de choix pour la recherche des coli indologènes.

Lieut.-Col. C. H. H. Harold (London) : *The Microbiology of the London Water Supply.*

The periods of greatest pollution in the rivers are times of flood when the greatest coli abundance is associated with the highest colony counts.

Qualitatively, river-derived waters reflect the microbiological characteristics of the river throughout the stages of purification. In association with storage and filtration there is an increasing ratio of non-typical organisms to typical organisms as we pass through these stages of purification and also, within each stage, in the summer as compared with the winter months.

Quantitative routine results for the past 28 years show that, in the River Thames and Thames-derived waters, the coli content and colony counts are lower in the spring compared with the winter and autumn quarters. This improvement is maintained in the summer in the case of the raw waters, but, owing to high rates of filtration in the summer, consequent upon increased demand, a rise in the coli and colony counts occurs in filtered waters.

Consequent upon storage and filtration, the purity of River Thames water is enhanced about two-thousand-fold with regard to the coli content, a hundred-fold as regards the agar count, and over a hundred-fold in the gelatine count.

Throughout the year the dominant pollutive index is typical *Bact. coli* with a relative increase in atypical organisms and a definite increase in gelatine liquefiers of the Proteus type during the summer months.

Direct plating technique reveals 60 per cent. of organisms isolated from raw Thames water to be typical *Bact. coli*. The second

most prevalent type is an Intermediate (indole negative, citrate positive, methyl red positive, Voges-Proskauer negative), present to the extent of 18 per cent. In raw waters 3·5 per cent. are of the aerogenes-cloacæ group, and they account for only 1·1 per cent. in stored waters. By enrichment methods only 2·4 per cent. of aerogenes-cloacæ strains have been isolated, mainly from filtered waters and the majority are indole negative. There appears to be no increase in their prevalence in summer.

Arising from these special investigations differences appear in the ratios of coliform types existing in reservoirs having prolonged storage and a large bird population.

Investigations in England show that conditions of growth of indicators of pollution differ from those obtaining in tropical or sub-tropical areas, where multiplication of the coli-aerogenes group of organisms must be specially considered. Experience at home and particularly in the East leads to the conviction that although *Bact. coli* is undoubtedly the most certain and dependable index of water pollution, the claims of atypical organisms including *Bact. aerogenes* and the subsidiary indicators, *Clostridium welchii* and the fæcal streptococcus cannot be ignored.

Prof. R. Kolkwitz (Berlin): *Zur Mikrobiologie der Wasserversorgungsanlagen.*

Schwefelbakterien sind bereits zusammenfassend gründlich behandelt worden, doch sind neuerdings Zweifel darüber entstanden, ob Schwefelwasserstoff die einzige Quelle der Energiegewinnung ist. Es scheinen auch andere spezifische Schwefelverbindungen oder feinst verteilter kolloidaler Schwefel diese Dienste zu leisten.

Eisen- und Manganbakterien sind gleichfalls zusammenfassend behandelt, doch bedarf es noch weiterer Studien über den Bedarf an Sauerstoff und über die Nachhaltigkeit, mit der die gelösten Eisen- und Manganverbindungen gebunden werden.

Sphærotilus, dessen Ernährungsweise bekannt zu sein schien, stellt uns neuerdings weitere Aufgaben, besonders über den Nährwert mancher zyklischen Verbindungen. Jedenfalls spielen nicht nur Kohlenhydrate und Eiweissverbindungen für seine Ernährung eine Rolle.

Kieselalgen erscheinen in manchen Wasserwerken als Fossilien im Wasser und mahnen dadurch zur Vorsicht bei der Beurteilung seiner Beschaffenheit.

Wasserblüten können durch Riechstoffe in Talsperrenwässern das Wasser nachteilig beeinflussen. Sie lassen sich bei rechtzeitiger Anwendung chemischer Mittel besser bekämpfen als früher. Vielleicht führt auch die Behandlung des Wassers mit Ozon zu nachhaltigeren Erfolgen als bisher.

Zisternen erfordern eine besonders geschickte Behandlung zwecks Speicherung und Reinerhaltung des Wassers. Das Studium ihrer Biologie verspricht besonders in wärmeren Ländern wesentliche

Aufschlüsse, für die Biologie selbst und für Wassertechnik (erdige Zersetzung der Sedimente).

Sandfilter in wärmeren Ländern regen zu Vergleichen mit der Zusammensetzung der oberen Filterschichten in kühleren Wässern an. (Nil, mitteleuropäische Flüsse, usw.).

Desinfektionswirkungen (durch Chlor, Kupfer, Silber u.s.w.) bieten charakteristische Absterbeerscheinungen und sind geeignet zu Beobachtungen über die feinere Pathologie der Zellen in Beziehung zu spezifischen, chemischen Stoffen.

Wo von *Sphærotilus* die Rede ist, könnte eingefügt werden: dass er vielleicht auch von Cyanverbindungen leben kann.

Dr. R. W. Butcher (Alresford, England) : *The Value of Microflora as indicating the Composition of Natural Waters.*

It is well known that the presence of certain micro-organisms in water may be used for indicating its composition though, except for the *Bact. coli* group, lack of data make them at the moment of small value.

For example, *Sphærotilus natans*, commonly known as sewage fungus, occurs in running but not in still water, when there is present in the water organic matter from sewage, organic wastes from food-factories, or carbohydrates. *In vitro, Sphærotilus* grows very well in weak solutions of meat extracts, protein, amino-acid but not in ammonium salts or nitrates. If carbohydrates be added to the nitrate medium there is vigorous growth. This may explain why effluents containing little but carbohydrates will cause growth when added to river-water which always contains nitrates.

A robust form of the alga *Cladophora glomerata* is almost invariably found in running water which has been polluted but has repurified itself. The presence of this alga seems to be determined by the quantity of natural salts produced by the decomposition of the sewage.

For several years algal growths on glass slides have been collected and estimated from a number of rivers of different composition. The date presented shows marked correlation between the composition of the waters and the algæ growing therein.

In a New Forest stream with a *p*H of 6·0 a hardness of less than one part in a 100,000 and a nitrate content of about 0·02 parts, the dominant algæ are the diatoms *Tabellaria flocculosa, Eunotia arcus* and *Eunotia lunaris*. As the hardness increases downstream *Tabellaria* disappears but it takes a smaller quantity of calcium to inhibit its growth in running water than it does in still water; this may be because in the former, the solution of food substances and salts is being continually renewed by the current.

In a hard water such as the Itchen with a *p*H of 7·5, a hardness of 22·0 and a nitrate content of about 0·035, the normal growth consists of *Cocconeis placentula, Chamæsiphonopsis* and *Ulvella frequens*.

In the Tees also the distribution of algæ is correlated with the amount of organic matter and mineral salts brought in by sewage and drainage. In the uppermost portion the algal-growths consist mainly of *Achnanthes microcephela* and *Chætopeltis megalocystis;* where there was sewage pollution this was replaced by *Cocconeis* and *Chamæsiphonopsis.* On repurification the former community at first returned but further downstream the effect of the sewage and drainage seemed to be cumulative as the two communities were there seen side by side.

Other examples might be cited such as the Bristol Avon where milk wastes produce a very heavy growth, particularly in *Navicula viridula, Stigeoclonium tenue* and *Nitzchia amphibia,* and it seems that a study of the algæ, particularly those with limited distribution, will be of great value in indicating the composition of a water supply when the causal factors are ascertained.

Dr. B. E. Proctor (Cambridge, U.S.A.) : *The Microbiology of the Upper Air.*

With the increasing use of air-craft, facilities for co-operative studies of the microflora of the upper air are more readily available. In the United States and possibly other countries daily high altitude meteorological flights are being made from a number of stations to heights of 15,000 feet or more for weather forecasting data.

The dispersion and transmission of air-borne micro-organisms, pollen and dust are of significance in agriculture, health and biology. The types, relative incidence and survival of micro-organisms in the upper air strata deserve further study and attention.

An investigation of certain phases of upper-air microbiology has been conducted at the Massachusetts Institute of Technology for several years, using an apparatus known as a bio-aercollector. This device, sterilized previous to each flight, is capable of making six collections in one flight, each at a different altitude. For operation during flight it requires only the turn of a knob by the pilot, so that an additional person for taking the collections is unnecessary. A system of subsequent laboratory technique enables visual microscopic inspection or a micro-photographic record of all biological and dust specimens under sterile conditions, so the viable micro-organisms collected may later be cultivated in suitable media, enumerated and sometimes identified.

Collections made during the course of more than 150 aeroplane flights have indicated the common presence of viable bacteria, moulds and yeasts in the upper air strata at altitudes up to and exceeding 20,000 feet during all seasons of the year.

No definite relations have been observed between temperature, relative humidity, precipitation or air masses and the numbers or types of micro-organims found in these flights. At the higher

levels the number of micro-organisms is usually somewhat diminished but the reverse has been observed in some instances.

From the bacterial collections made above 10,000 feet the *Bacillaceæ* have been isolated most frequently, although numerous species of *Bacteriaceæ* and *Coccaceæ* have also been identified.[1,2] Among the moulds collected, the Aspergilli and Penicillia have been more frequently found. Viable Actinomyces and yeasts have also been collected but the occurrence of these forms is less common.

Pollens have also been isolated at high altitudes, using the same apparatus.

The interesting possibilities of fundamental information which might be derived from numerous research stations using some standardized technique and preserving photographic records of collected specimens are manifold. The fact that planes primarily for other purposes must make altitude flights each day, should stimulate other investigators to enter this field and further the progress of this type of study on numerous fronts.

Mr. W. F. Wells, Prof. G. M. Fair, Dr. Mildred W. Wells and Mr. A. B. Whisler (Harvard Univ., U.S.A.) : *Viability of Micro-organisms in Air.*

This paper summarizes studies that have been in progress at Harvard University during the past five years. Using a simplified technique of air sampling which employs an air centrifuge to precipitate micro-organisms from a measured volume of air directly on to a solid nutrient medium or into broth or a saline solution, studies have been made of the viability of micro-organisms in air. These include (1) the sampling of air infected artificially by atomizing into the air a liquid containing pure cultures of micro-organisms and (2) the sampling of air in occupied spaces, such as school rooms, clinics, operating rooms, hospital wards, theatres, factories and experimental chambers. Droplets less than 0.1 mm. in diameter when expelled into the air evaporate to dryness in a shorter time than required to fall the height of a man. The nuclear residues drift in the air for long times and long distances with the slightest air currents. The viability of the microbiological population of these nuclear residues is appreciable. *Pneumococcus* Type I, *Corynebacterium diphtheriæ*, *Streptococcus hæmolyticus* and *Streptococcus viridans* have been recovered from the air of a still chamber 48 hours after artificial infection of the air ; typical intestinal bacteria disappeared in 12 hours. Dysentery phage and the virus of human influenza has been recovered from artifically infected air. Typical naso-pharyngeal organisms have been recovered from the air of occupied spaces.

The effect upon viability produced by humidity, ozone, a spray disinfectant and ultra-violet light has been studied. The viability

[1] Proctor, B. E., *Proc. Amer. Acad. Arts & Sc.*, 1934, **69**, 315.

[2] Proctor, B. E., *J. Bact.*, 1935, **30**, 363.

of the organisms so far employed decreases with decreasing humidity. The destructive power of ultra-violet light is of appreciable magnitude even at considerable distances and is proportional to the light energy intercepted by the living organisms. The effect of ultra-violet irradiation has been determined for a number of different types of organisms.

Lieut.-Col. C. H. H. Harold (London).

The subject of Prof. Fritsch's communication is one of increasing importance to the Metropolitan Water Board, which will shortly hold reserves of 30,000 million gallons in storage, or the equivalent of over 22 days total average Thames flow. Successive outbursts of algal growths have appeared, particularly in Queen Mary Reservoir (6,750 m.g. capacity). In the past, following deoxygenation, a billowy mass of *Oscillatoria* appeared, which rendered it unusable for months. It has recently sustained a pulse of *Fragilaria*, density counts of 2,500 per ml., having an estimated total weight of wet growth of 10,800 tons with 90 tons removed by filtration daily and the weight of dry silicate being 68 tons. On the New River protracted pulses of the flagellate *Chlamydomonas* which have the faculty of passing the filters, have appeared, and as a result complaints have been received from mineral water manufacturers, brewers, tea blenders, Zeolite manufacturers, users of softening plants, etc., about the green colour of the water, its hardness and silica solvency.

Prof. Fritsch has laid great emphasis on the importance of the chemical content of water, and the Thames and Lee are practically never deficient in this respect.

Photo-micrographic records of over 25 years show that certain species, particularly *Oscillatoria, Fragilaria, Asterionella* may show a variable prevalence throughout certain years, but in the main the majority of types would appear to have an inherent habit of selective periodicity. The sunshine records and temperature also seem to bear some relationship to the spring maxima of the green algæ.

With reference to Dr. Butcher's communication, heavy growths of *Tabellaria* have appeared in the hard waters in the reservoirs, and it can hardly be termed a soft water diatom. The suggestion that fungi do not develop in quiescent waters is not supported by fact, since the Queen Mary Reservoir had to be taken out of service in the second summer of the drought, on account of de-oxygenation and a massive invasion of *Cladothrix dichotoma*.

Prof. A. M. Buswell (Urbana, Illinois).

It is encouraging to hear Col. Harold say that he attaches importance to *Bacterium aerogenes*. His high standards are also reflected in the palatability of the London water.

Prof. Kolkwitz's observation on the growth of *Sphærotilus* at high temperature is paralleled by the experience of Dr. Hatfield in the treatment of warm stack waste by the activated sludge process.

It is unfortunate that more work of the type described by Dr. Butcher is not carried out.

Prof. Jan Smit (Amsterdam).

The development or death of *Bact. coli* in natural waters is entirely dependent upon the chemical composition of the water, so that the behaviour of *Bact. coli* can be predicted if the composition is known.

Dr. A. C. Gardiner (London) gave a brief account of the routine chemical analyses carried out by the Water Examination Department of the Metropolitan Water Board.

He stated that an attempt was being made to estimate the quantities of phyto-plankton and to ascertain the qualitative composition. Observations would be needed for many years before these data might be of service to allow us to forecast the times of appearance and disappearance of algal phases.

Reference was made to the occurrence of a period of de-oxidation in 1935 (already mentioned by Lt.-Col. Harold), and to the appearance of a similar condition in 1936. The latter phase suddenly disappeared, following, in all probability, the onset of a week of colder, wet and windy weather.

Prof. J. W. Bigger (Dublin).

I have found that a variety of coliform bacilli, including typical *Bacterium coli*, are capable of multiplying in water. Among waters permitting growth were those of Dublin and London (Thames and Lee). An inoculum of 100 per c.c. may, in a few days at 37° C., lead to the presence of several hundreds of thousands per c.c. In these experiments waves of growth were observed. The best growth was obtained in waters which had been heated, filtered through Chamberland filters or otherwise treated; but growth can occur in natural raw water. This may explain the finding of large numbers of coliform bacilli in a water from a source free from contamination.

Lieut.-Col. C. H. H. Harold (London).

In reply to Professor Bigger's comments, nearly twenty years ago, when investigating cholera outbreaks in India, I was able to satisfy myself that under tropical conditions the coli-aerogenes

group was capable of growth in wells. Multiplication does not appear on the works in England. The Queen Mary Reservoir in the winter may carry an avian population of upwards of one million birds providing an addition of one coliform organism per 3 ml. daily.

In consequence of Professor Bigger's results, the problem of after-growths was investigated in the Board's laboratories, and multiplication of these organisms appeared to be related to the efficiency of the disinfection of the water prior to seeding, e.g. (1) Boiling, (2) Chamberland filtration, (3) Chlorination, (4) Ozonization, (5) Works filtration—water disinfected by this process did not support multiplication.

The possibility of the detrimental effect of normal saprophytes appeared. Results with naturally carbonated Kent Well water were discordant, and in general the results appeared to be affected by a variety of inconsistent and unknown factors.

Prof. F. E. Fritsch (London).

Professor Fair referred to the possible relation of algal phases to the turnover that occurs in large bodies of water. In the English lakes, however, this does not by any means always coincide with the periods of algal maxima, and it appears evident that other factors are involved.

As regards the possible effect of backwaters and tributary streams on the plankton of a river, such evidence as is available is altogether negative. Mr. C. H. Rice had recently investigated the influence of the phytoplankton of backwaters and tributaries on the composition of Thames plankton and, with few exceptions, no influence of the plankton of the tributary on that of the main stream could be recognized, dominant forms in the former playing no rôle in the Thames below the point of influx. On the other hand, in some cases a different, presumably chemical effect was noticed, since forms that played a conspicuous part in the plankton of the main river above the point of inflow of the tributary were much rarer below that point.

Dr. R. W. Butcher (Alresford).

The outfall of a reservoir contaminated with *Asterionella* was traced to a river three miles down. The *Asterionella* disappeared immediately, and the water picked up diatoms from the river bed instead.

The change of algal growth due to flood referred only to hardness and *Sabellaria*. The first effect of a flood in the small rivers of England is to sweep all the bottom and back-water algæ into the plankton zone, increasing it enormously, but this increase is short-lived, and the numbers return to normal within a day.

Prof. R. Kolkwitz (Berlin).

Die gute Ozon-Methode ist leider noch teuer, aber die Firma Siemens (Berlin) ist eifrig bemüht, sie zu verbilligen. Ozonbehandlung kommt auch für Schwimmbäder in Betracht. *Sphærotilus* wird in Deutschland beseitigt durch biologische Reinigung der Abwässer (vorwiegend Oxydation) z.t. auch durch Ausfaulen. Bei kurzen Flüssen verhindert Chlorzusatz (auch Cl+Cu) die Entwicklung.

DISCUSSION *on* THE MICROBIOLOGY OF PERISHABLE FRESH FOODS, OTHER THAN MILK AND MILK PRODUCTS, AND THE MICROBIOLOGY OF CANNED FOODS, OTHER THAN MILK AND MILK PRODUCTS

Chairman : Dr. L. H. Lampitt (London).

Dr. L. H. Lampitt (London), **Opener** : *The Microbiology of Perishable Foodstuffs.*

I have been requested to open the session by some general remarks on the microbiology of perishable foodstuffs. The study of micro-organisms has in the past had a decided medical bias, in other words, the chief stress has been on the pathogenic type of organisms, but during the last generation a broader appreciation of the questions involved has become evident. Moreover the purely morophological aspect has given place, gradually, to a point of view which is distinctly chemical. This change is to be welcomed because it is only as the knowledge of the chemical changes wrought by micro-organisms are understood that an intelligent control can be effected and control as far as food is concerned is of primary importance.

It might be concluded from the use of the word " Perishable " that the object was to stress the importance of those micro-organisms which are concerned in the spoilage of food, but there is the other side to the picture—namely the economic utilization of micro-organisms in the production of food, the production of beer and wine, the fermentation of cocoa pods, the manufacture of cheese and sour milk products, the baking of bread, the pickling of meat; and no survey of the activities of moulds and bacteria would be adequate were no mention made of these world-wide uses.

The main stimulus to work on the effects of micro-organisms can be attributed to modern conditions of urbanization of the population of the globe, whereby those who produce food are more definitely segregated from those who consume it than ever before, and this massing together of men and women necessitates transport of food from the site of its production to the place of utilization, the whole being complicated by the fact that not only the epicure, but the average man, requires luxuries of every clime daily on his table.

Preservation is therefore essential and consequently those actions which tend to degrade the value of the article, must be arrested or retarded. Such actions may, however, be divided into two groups :—

1. Fermentative and putrefactive changes due to micro-organisms.
2. Changes, such as drying, oxidation, rancidity, etc. due to other factors.

It must be remembered that until comparatively recent times these different causes of food spoilage have been confused. The theory that the "rancidity" of milk powder was due to micro-organisms was generally held until ten years ago, whereas now it is recognized as belonging to the second class of purely chemical change, a type of change which from the health point of view is relatively unimportant; the food value of the product is not necessarily reduced, although the flavour and palatability of the material may be adversely affected.

Excluding the "constructive" work of organisms such as I have mentioned, the most important concern is the avoidance or control of undesirable changes.

To preserve food in wholesome condition from times of plenty against those of scarcity or famine has been a serious problem for men from time immemorial. Primitive man found that meat and fish could be preserved by drying or smoking—methods which are in use to-day, although, with the development of science and the resulting increase in the knowledge of the causes of spoilage and of the principles involved in preservation, the treatment is no longer entirely empirical.

The necessity for the protection of food against contamination by micro-organisms is now well realized. The employment of hygienic methods of treatment, absolute cleanliness in preparation, suitable methods of treatment to destroy micro-organisms, and protection of food during transport and storage by means of suitable packaging to avoid recontamination, have become essential practices in the Food industry.

The general methods of preservation employed in practice fall roughly into two groups :

1. Those which restrict the development of organisms present in the food.
2. Those which aim at the destruction of all or most of the organisms present.

There are obviously many ways by which the desired object can be achieved. Those which I have included in the first group are broadly, cold storage, gas storage, drying and pickling.

Preservation by destruction of the organisms present—the second group—is effected by (*a*) heat, as in sterilization and pasteurization, and is usually combined with subsequent adequate protection by means of airtight containers to obviate re-contamination, or by (*b*) Antiseptics or chemical preservatives.

May I refer briefly to some practical considerations which are of prime importance to the food manufacturer?

Any method of preservation selected for a particular food must fulfil the following requirements:

(1) It must be effective in preventing the growth of those micro-organisms which may produce spoilage in the food concerned.

(2) It must produce minimum alteration in the food, or, if some alteration is unavoidable, the product must be no less palatable and attractive than the original food.

(3) It must not make the food subject to attack by micro-organisms which do not normally affect the untreated food.

In conclusion, I would stress that our knowledge of the subject is still relatively scanty, that many of our methods are as yet empirical, and that the number of problems awaiting solution are legion.

Prof. W. Schwartz (Karlsruhe): *Die mikrobiologischen Grundlagen der Konservierung von Fleisch und Fisch durch Kälte.*

Bakterien und Pilze sind die wichtigsten Ursachen für das Verderben der Lebensmittel. Bei kühl gelagertem Fleisch und Fisch wird die Zersetzung von Bakterien durchgeführt. Ihre zahlenmässige Erfassung ist wichtig, weil der Anfangskeimgehalt die Haltbarkeit beeinflusst und weil Beziehungen zwischen Oberflächenkeimgehalt und Frischezustand bestehen, sodass der Keimgehalt je cm^2 Oberfläche als Masstab für den Einfluss verschiedener Lagerungsbedingungen auf die Haltbarkeit dienen kann. Die Vorgänge bei der Tiefeninfektion bedürfen noch einer besonderen Untersuchung. Beim Fleisch geht der Anfangskeimgehalt auf die Infektion bei der Schlachtung zurück; bei Fischen erfolgt die primäre Infektion beim Fang. Es ist gelungen, die Infektionsvorgänge an Bord des Fischdampfers aufzuklären und die Vermehrung der Bakterien während des Transports der Fische auf dem Dampfer zu verfolgen. Wichtigste Infektionsquelle ist der Darminhalt der Fische. — Die konservierende Wirkung niederer Temperaturen beruht auf der Ausschaltung kälteempfindlicher Mikroorganismen und auf einer mit sinkender Temperatur zunehmenden Hemmung der kryophilen Keime. Das Ziel ist, die Hemmung so zu verstärken, dass die Wachstumskurve (Oberflächenkeimgehalt in Abhängigkeit von der Lagerdauer) den für die Haltbarkeit kritischen Grenzwert von etwa 10^7 lebenden Keimen je cm^2 nicht mehr erreicht. Da dies bei einfacher Kühllagerung nicht gelingt, müssen kombinierte Verfahren entwickelt werden, durch die der Anfangskeimgehalt herabgesetzt und die Kältewirkung während der Lagerung verstärkt wird. Durch Vergleich unterkühlter und gefrorener Fleischproben bei $-2°$ C. gelingt es, den Einfluss des Gefrierens auf das Wachstum der Bakterien zu ermitteln. Die Hemmung der gewöhnlichen

Fleischbakterien nimmt sofort stark zu, vermutlich infolge der durch den Wasserentzug bedingten Erhöhung des osmotischen Druckes im Fleischsaft. Einige der auf Fleisch vorkommenden Pilze sind gegenüber einer Druck-Erhöhung im Substrat weniger empfindlich, sodass sie als Schädlinge auf Gefrierfleisch auftreten. Herabsetzung der relativen Feuchtigkeit der Luft hat mikrobiologisch einen ähnlichen Einfluss wie das Gefrieren, da sich zwischen dem Wasserdampfgehalt der Luft und dem Wassergehalt des Substrates ein Gleichgewicht einstellt. Das Feuchtigkeits-Optimum der Pilze und Bakterien liegt nur wenig unterhalb 100 prozent relativer Feuchtigkeit.

Mr. E. L. Crossley (Yeovil, England): *Bacteriological Control in the Manufacture of Meat and Fish Pastes.*

The bacteriological problems involved in the production of meat and fish pastes have been studied; the method of processing employed was preservation by heating in hermetically-sealed glass jars.

Since 1932 bacteriological examinations have been made of 14,365 jars of paste from various sources. Of these 12,624 (87·9 per cent.) were sterile; 1,726 (12·0 per cent.) contained living spores of various common aerobic bacilli, mainly *B. subtilis* and *B. megatherium*, and 15 jars (0·1 per cent.) contained spore-forming anaerobes of the *Cl. sporogenes* and *Cl. putrificus* types. An extremely heat-resistant strain was isolated.

Experimental and practical evidence was obtained which showed that the anaerobic bacilli were the main cause of spoilage —an uncommon defect—though development of these organisms is irregular. Aerobic spore-forming bacilli when present alone could not bring about spoilage within a sealed jar, but their presence indicated that the margin of safety was very small.

The chief causes of possible failure encountered were :—

(1) Imperfect sealing of the jar.

(2) Imperfect processing of the jar, due to :—

(*a*) Excessive numbers of bacteria in the paste before processing, derived either from the raw materials or added as a result of faulty methods of preparation. Careful bacteriological tests of all materials and equipment were found highly desirable.

(*b*) Employment of too low a processing temperature or too short a heating period. Heat penetration to the centre of the jars should be confirmed by the use of a suitable thermo-couple. Another satisfactory method employed was experimental processing of jars of paste heavily inoculated in the centre with cultures of *Cl. sporogenes* and *Cl. putrificus*.

(*c*) Uneven distribution of steam in the retorts, which could be discovered by placing maximum thermometers in various situations.

(*d*) Failure of the human operator or automatic regulator, or fluctuations of steam pressure.

For satisfactory control it was found *essential* to make bacteriological examinations of representative samples drawn from every batch processed. A new medium was developed containing skim milk, peptone and bromocresol purple, and a very heavy inoculum of paste was necessary. The method of testing worked out enabled both anaerobes and aerobes to be detected very simply, rapidly and accurately without the use of elaborate apparatus.

Prof. F. W. Tanner (Urbana, U.S.A.): *The Microbiology of Canned Foods.*

Great quantities of foods of all kinds are preserved by heating in glass and tin containers. Factory canners to-day use the most recent information from bacteriology, chemistry and physics. All steps involved in canning are important, but not equally important from the bacteriological standpoint. While preliminary operations such as careful washing, sorting and removal of defective portions markedly influence the bacterial load which will have to be handled later by the heat-process, most investigations have related to the heat treatment or "process" which must be depended upon to make canned foods keep well and be safe. Process times and temperatures were formerly obtained by the trial and error method. To-day they are based on results of such investigations as:—

(1) Commercial experience of the industry.

(2) Determination of the rate of penetration of sufficient heat to destroy spores of bacteria to the centre of the container of various sizes and shapes under the different conditions of processing.

(3) The heat resistance of suspensions of bacteria which may spoil the food or make it unsafe; among the latter is *Clostridium botulinum*.

(4) Results from processing in different ways of experimental packs inoculated with known spoilage bacteria. The inoculum used is usually a typical spoilage organism, spore suspensions of which are standardized to yield a heat resistance comparable to that of *Clostridium botulinum*.

Selection of process times and temperatures should come, therefore, only after much careful scientific work, a fact not realized by some who have tried to pack new products and especially many who disseminate advice on canning in the home.

Spoilage bacteria should be carefully studied. Their heat resistance should be known in order that they may be used in inoculated experimental packs. Thermophilic bacteria are among

the most troublesome spoilage bacteria which plague the canner. They have been endowed by nature with just those characteristics which make them a menace in the cannery. Very heat resistant spores are formed and they grow anaerobically at high temperatures.

Canned foods are not always sterile, the degree of incidence of viable spores depending on the food product. Since heat treatment applied to canned foods does not destroy all spores, although the foods remain in good condition and fit for food, the term "processing" was introduced in place of "sterilization." Later the expression "commercial sterility" was introduced to express the bacteriological status of foods which had been subjected to heat treatment, but which still contained viable spores. Some of the viable bacteria in certain foods are coccus forms which have not ordinarily shown appreciable heat resistance. Why they are able to survive processes which ought to kill them, is probably not known. One possible theory is that they survive in the fat where there may be little water. It is well known among bacteriologists that dry bacteria are killed with difficulty. Contaminated cultures cannot account for these observations.

Factory canned foods packed in America have not caused botulism for over ten years. Several outbreaks have been caused by imported foods. Home-canned foods in America are causing each year some fifteen or twenty outbreaks. Until the procedures recommended to the home makers are based on sound scientific data, and until the uninformed home-maker can be taught to use properly some of the necessary apparatus, these outbreaks will continue.

Mr. D. H. F. Clayson (London): *The Preservation of Cooked Food for Limited Periods.*

The term "preservation," when applied to food, is generally considered to involve such processes as hermetical sealing, drying or curing, but food is frequently stored for short periods without such treatment. How long food can be stored depends largely upon the extent to which it is subject to, or exposed to, microbial attack, but, apart from investigations following food-poisoning outbreaks, there is little published work concerning the application of micro-biological methods to this question.

Raw foods are contaminated to a variable extent by micro-organisms liable to cause spoilage, either in the raw or cooked conditions. Usually one has to treat the cooking as a means of sterilization, but it must be realized that the primary object of cooking is to make the food palatable and that frequently it is not practicable or desirable to make the heat treatment sufficiently drastic to ensure absolute sterility. Fortunately it is a general rule that the more heat-resistant organisms do not commence to grow in most cooked foods until after an appreciable time interval, the lag phase frequently extending to 36 to 40 hours at 20° C. in

the case of sporing aerobes and heat resistant Micrococci. Organisms of lower heat resistance, such as Staphylococci and *Streptococcus fæcalis* may produce appreciable spoilage in 12 to 14 hours and members of the Coli-Aerogenes group in an even shorter period.

During roasting or frying, meat or fish may not be heated above 60° C. at the centre, consequently many organisms, if present there, would survive the cooking treatment. Meat or fish muscle or fatty tissue, however, is only infected at the cut surfaces, if in good condition, consequently thorough heat penetration is not necessary. In the case of products composed of chopped or minced meat moulded into a compact mass it is necessary to reduce contamination as far as possible and to control the cooking so that the material is thoroughly heated without deleterious effect upon palatability.

Spoilage may frequently be caused by recontamination after cooking. The utensils used, therefore, should be such that they can be repeatedly cleaned and sterilized without becoming pitted or rough. Refrigeration may be applied to retard the growth of surviving organisms or chance contaminants. Wrapping will reduce recontamination, but impervious wrappers may enclose air of high humidity, if the product is of high moisture content, and consequently favour the growth of surface contaminants. Excessively pervious wrappers, however, may allow drying to occur at the surface with a deleterious effect upon appearance and palatability.

In conclusion, the preservation of cooked food, without hermetical sealing, depends largely upon suitable control of cooking, upon maintaining any surviving organisms in the lag phase, and preventing recontamination.

Dr. D. J. Tilgner (Warsaw), reminded the meeting that a number of contributions were made during the last Congress of Refrigeration, held in Holland a month ago, on the subject of the microbiological content of refrigerated merchandise prior to introduction into the factory for manufacturing purposes.

It should be remembered that economic aspects influenced the introduction of scientific findings into practice. Very often commerce preferred to take some risk instead of making heavy investments to attain the 100 per cent. ideal of microbiological security. The main and cheapest weapon of the food industry against microbiological threats was cleanliness.

With regard to Professor Schwartz' contribution, not only the numbers of micro-organisms, but the different kinds and their influence must be taken into consideration. In this respect much remained to be studied, since some species produced highly dangerous changes, while others had harmless or unimportant effects.

It was the speaker's experience that home preservation of food played an unimportant rôle on the European continent. The end-product, made without any adequate understanding of fundamentals, lacked firmness, colour and quality as compared with the

manufactured article. Further, there was no assurance that the product was free from contamination with *Clostridium botulinum*. Nevertheless, large numbers of pressure-cookers, amounting to F. 10,000 in value, had been sold in Germany alone. A similar trend was now observable in Poland.

Dr. H. Damm (Kiel).

Echte Streptokokken können in Konserven, die über 100° C. erhitzt worden sind, nicht vorkommen. Es besteht aber die Möglichkeit, dass Sporenbildner nach der Erhitzung ein Streptokokkenähnliches Aussehen annehmen und sogar ihr Sporenbildungsvermögen verlieren. In diesem Zusammenhange verweise ich auf die Arbeit von Janke aus der Kieler Forschungsanstalt.

Prof. W. Schwartz (Karlsruhe).

Der Oberflächenkeimgehalt kann als Mastab für die Vermehrung der Bakterien im Laboratoriumsversuch dienen, wenn es sich darum handelt, die Abhängigkeit der Vermehrung von einzelnen Aussenfaktoren festzustellen. Das Interesse an der *Art* der Bakterien tritt hierbei gegenüber der *Zahl* zurück. In der Praxis muss natürlich die *Zusammensetzung* der Bakterienflora berücksichtigt werden.

Mit Rücksicht auf die Verhältnisse in der Praxis ist es ferner wichtig den Vorgang der Tiefeninfektion histologisch zu verfolgen. Versuche in dieser Richtung haben wir in Karlsruhe in Angriff genommen.

Dr. R. B. Haines (Cambridge).

Microflora on chilled beef are composed chiefly of *Achromobacter* and *Pseudomonas*. These organisms have an optimum temperature of 37° C. and do not as a rule grow at refrigeration temperatures.

Mr. P. M. Short (London), questioned whether manufacturers paid sufficient attention to the duration of storage of fresh fish, game, etc., before canning.

Mr. E. L. Crossley (*Yeovil*).

Heat-resistant cocci are found in tinned anchovies and tinned sardines, but, in general, care must be taken to ensure that cocci are not due to an air leakage in the container.

It is important to carry out both laboratory and factory trials of processing. Sometimes the heat treatment required for the factory product is less than in laboratory media. Much unsound advice on home preservation is given in the English press. Routine

control at the factory is essential, since the bacteriologist is able to check any errors at once.

Details of medium mentioned in my paper are as follows :—

Skim milk plus 1 per cent. peptone, and brom-cresol purple as indicator. Use a large inoculation of paste and incubate for three days at 37° C.

Dr. L. H. Lampitt (London).

Most large food factories have definite laboratory control of their " pack." " Cooling " is also usual.

JOINT DISCUSSION *with* SUBSECTION 3 *on* THE PROCESS OF DECOMPOSITION OF PLANT REMAINS IN SOIL, MANURE AND COMPOST HEAPS

Chairman : Dr. C. Stapp (Berlin).

Prof. G. Ruschmann (Landsberg a/Warthe), **Opener.**

Alle organische Substanz unterliegt einer raschen Zersetzung, die, soweit es die Landwirtschaft interessiert, auf dem Stalldünger und Komposthaufen beginnt und im Boden ihre Vollendung findet. Dem Abbau steht eine ebenso schnelle und starke Neubildung organischer Substanz gegenüber, deren Menge mit rd. 35 Bill. kg. jährlich berechnet wird. Dies ist nur dadurch möglich, dass die Kleinlebewesen die Zersetzung der gebildeten Massen unter den verschiedensten Bedingungen vollführen und damit den Kreislauf der Nährstoffe sicherstellen. Obwohl diese Tatsachen praktisch und theoretisch von gleich grosser Bedeutung sind, erkennt man den Nutzen und die Lehre von den Kleinlebewesen, die uns gestattet, die Vorgänge zu regeln, vielfach nicht an.

Als Zersetzungserscheinung muss schon die Veratmung organischer Substanz in lebenden Zellen nach dem Tode eines höheren Organismus gelten. Auch in abgestorbenen Geweben und Zellen bewirken die Pflanzenenzyme häufig einen weitgehenden Abbau hochmolekularer Verbindungen (Eiweisstoffe). Viel schneller und tiefgreifender zersetzt sich aber die organische Masse unter dem Einfluss der Kleinlebewesen. In dem einen Fall sind die Vorgänge mehr als Gärung und Fäulnis, in dem andern Fall mehr als Verwesung, d.h. als Mineralisierung der organischen Substanz aufzufassen. Durch Gärung, Fäulnis und Verwesung entstehen in und auf dem Boden jährlich ungefähr 60 Bill. kg. CO_2, sodass derselbe mit einem grossen Gärbottich verglichen werden kann. Je wärmer und besser durchlüftet der Boden ist, je günstiger der Feuchtigkeitsgehalt und die Reaktion sind, desto schneller und vollständiger vollzieht sich der Abbau. Dabei spielt das Nährstoffverhältnis in der organischen Substanz (C:N—und C:P—Verhältnis) eine grosse Rolle.

Ein wichtiges Zwischenprodukt der Mineralisierung ist der Humus, dessen Bildung und Zersetzung grosse praktische Bedeutung haben. Die damit zusammenhängenden Fragen, die heute wieder in den Vordergrund des Interesses gerückt sind, lassen sich aber nur biologisch verstehen. So wertvoll die Isolierung einzelner Humusverbindungen, Humussäuren, ist, so wenig kommt man damit dem Kern des Humusproblems näher. Denn Humus verbessert sowohl die chemischen als auch die physikalischen und biologischen Eigenschaften des Bodens und ist somit bestimmend für seine Fruchtbarkeit. Aus diesem Zusammenhang geht hervor, dass der Begriff des Humus, der teils dissimilatorischen teils synthetischen Ursprungs ist, sehr weit gefasst werden muss.

Die Stärke und Geschwindigkeit, mit der die Zersetzung organischer Substanz im Boden erfolgt, hängen von ihrer chemischen und biologischen Beschaffenheit ab. Durch einen höheren Gehalt an widerstandsfähigen Bestandteilen im Kompost und im natürlichen und künstlichen Stalldünger wird die schnelle und vollständige Zersetzung der organischen Substanz gehemmt, dafür aber die Humusbildung im Boden gefördert. Ein gut zersetzter, dunkel gefärbter Kompost wirkt in dieser Hinsicht anders als ein schlecht vergorener, heller Stalldünger. Es fehlt jedoch an einfachen und zuverlässigen Methoden zur Bestimmung des Humifizierungsgrades der organischen Substanz. Anderseits ist eine schnelle und weitgehende Zersetzung der organischen Substanz erwünscht, damit die Nährstoffe wieder in den Kreislauf zurückgeführt werden. Das Löslichwerden der Nährstoffe wirkt sich aber für das Pflanzenwachstum um so besser aus, je mehr Humuskolloide daneben entstehen, da hierdurch die Nährstoffe vor dem Auswaschen bewahrt bleiben. Auf alle diese Vorgänge hat die Bodenbeschaffenheit einen grossen Einfluss.

Zur Beurteilung eines organischen Düngers muss man seine Nährstoffwirkung, die eine Folge der Mineralisierung ist, von seiner Humuswirkung, die z.T. auf einer Neubildung organischer Suhstanz beruht, unterscheiden. Meist wird nur auf die 1- oder 2-jährige Wirkung des Düngers Wert gelegt, die hauptsächlich seinem Nährstoffgehalt zuzuschreiben ist. Humusbildung und Humuszehrung gehen langsam vonstatten. Da echte Humusstoffe für Mikroorganismen schwer angreifbar sind, ist es von diesem Standpunkt aus falsch, von einem leicht zersetzlichen oder mobilen und von einem schwer zersetzlichen oder Reservehumus zu sprechen. Ein guter Kompost enthält viel Humusstoffe und ist wegen des fortgeschrittenen Abbaues seiner organischen Substanz sehr keimreich. Auch ein systematisch heiss vergorener Stalldünger, Edelmist, weist einen hohen Humifizierungsgrad auf, ist aber sehr keimarm. Infolge der Wärmebildung und Wärmebewahrung finden Umwandlungen darin statt, deren erstes Stadium biologischer und deren zweites Stadium chemischer Natur ist. An dem Pasteurisierungserfolg kann man den richtigen Verlauf der Gärungen im Dünger ermessen.

Zur Humusbildung im Boden trägt besonders das in vielen Pflanzen gefundene, schwer zersetzliche Lignin bei, indem es mit Protein etwa in der Weise Verbindungen eingeht, dass die HN_2-Gruppe des Proteins mit der CO-Gruppe des Lignins reagiert. Das Produkt soll den Grundstoff aller Humusverbindungen bilden. Diese Ligninprotein-Theorie bedarf noch insofern der Stütze, als sich durch neuere Untersuchungen herausgestellt hat, dass das Lignin als solches nicht existiert, sondern erst bei seiner Bestimmung durch Einwirkung von Chemikalien aus Kohlehydraten entsteht, also ein Kunstprodukt ist. Trotzdem bedeutet es einen Fortschritt, dass es gelungen ist, aus Lignin und Protein unter Druck Verbindungen herzustellen, die sich genau wie natürliche Humusstoffe verhalten.

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Prof. I. Issatchenko (Moscow) [Communicated by Dr. Veillon] : *Etude de l'échauffement des débris tourbeux.*

1) L'échauffement des débris de tourbe entassés en pile est un processus biogène, certainement lié avec l'activité des microorganismes qui détruisent, en produisant de la chaleur, les matiéres organiques qui participent à la composition de la tourbe.

2) L'échauffement est favorisé par l'aération, une humidité déterminée par une réaction active favorable au développement des microbes.

3) Le nombre de microbes dans les débris de tourbe est considérable et au point de vue qualitatif sa flore est composée de bactéries, d'actinomycètes, de levures et de hyphomycètes.

4) Les couches superficielles des débris de tourbes sont plus riches en microbes que les couches intérieures et en général les débris de tourbe contiennent plus de microbes que la tourbe de laquelle ils sont extraits, car le traitement mécanique (l'aération) de la tourbe contribue à l'augmentation du nombre de microbes.

5) Le foyer de l'échauffement se trouve dans la couche mycélaire qui est disposée à la profondeur de 50–80 cm. à peu près et qui est composée en premier lieu de différents hyphomycètes.

6) L'élévation de la température dans la pile provoque des modifications de la microflore ce qui explique pourquoi le rôle dominant appartient successivement à différentes formes microbiennes dans les différentes périodes de la conservation de la tourbe. Pourtant, les hyphomycètes jouent un rôle plus grand dans les processus liés avec l'élévation de la température.

7.) Il faut discerner plusieurs stades en liaison avec le développement des microorganismes dans la pile :

1. Le développement de microorganismes et leur augmentation quantitative.

2. L'élévation graduelle de la température ± jusqu'à 35–40° C., c'est-à dire le début de l'échauffement de la tourbe.
3. Le développement croissant des formes thermophiles et de la désagrégation biogène des matières organiques qui commencent à se ralentir au moment où la température monte jusqu'à 70–80° C.
4. La formation par la haute température d'une masse graduellement carbonisée (demi-coke) provenant des produits de la transformation des différentes matières par les microbes, dont l'activité est arrétée vers ce moment.
5. L'inflammation comme suite de l'oxydation des masses carbonisées à condition de la pénétration de l'oxygène et comme suite l'élévation momentanée de la température jusqu'à 160° C. à peu près.

C'est ainsi qu'une phase suit l'autre dans les piles de débris de tourbe. Elles sont liées entre elles par des processus biogènes accompagnées de la formation de matières facilement oxydables et capables de s'échauffer jusqu'à la température de l'inflammation.

Il est encore difficile de dire quelles matières facilement inflammables se forment à la suite de processus biogènes d'abord et chimiques ensuite qui surgissent et se développent dans les piles de débris tourbeux dans certaines conditions. Néanmoins, on peut supposer que l'explication de l'inflammation qui serait la plus rapprochée de la réalité se trouve dans l'hypothèse suivante :

D'après Brown la désagrégation des hydrates de carbone, avec dégagement d'eau quand la température est relativement peu élevée aboutit à des liaisons non-saturées, de plus en plus riches en carbones. Ces matières poreuses se forment dans les couches profondes de la pile, c'est-à-dire dans les conditions d'anaérobiose relative et absorbent énergiquement l'oxygène dans les points non-saturés, quand ils sont accessibles pour l'air. Ceci amène une élévation rapide de la température qui monte justu'à l'inflammation.

L'action favorable du fer est lié avec ses propriétés de catalyseur. L'oxydation des liaisons non-saturées peut se réaliser aussi sans inflammation si les endroits où ils se forment sont d'une façon plus ou moins constante, en contact avec l'air et si l'oxydation se fait au fur et à mesure de la formation de ces liaisons. Du point de vue de cette hypothèse il nous était important d'établir dans notre étude la transformation des hydrates de carbone insolubles en hydrates de carbone solubles (sucres) qui pourraient servir de matériel pour la formation des liaisons non-saturées, capables de s'enflammer. Ceci fut le but définitif, tandis que la tâche la plus proche consistait, comme je l'ai déjà dit — d'établir le caractère biogène de l'échauffement des débris tourbeux en analogie avec ce qui a été effectué par moi et mes collaborateurs par rapport au grain déposé dans les élévateurs.

Prof. E. Melin (Upsala, Sweden) : *Decomposition of Some Types of Forest Litter.*

The factors which influence the rate of decomposition of the litter are both interior and exterior ones. The former include the chemical composition of the dead residues, the latter the climate and soil factors, as well as the composition of the microbial population. In the present study an investigation has been made of the action of these factors on the rate of decomposition of the litter. The material used was chiefly newly-fallen leaves which had been collected in the autumn. About fifty different forest plants have been investigated, the rate of decomposition being judged by the production of CO_2 and the quantity of organic matter decomposed during four to five months at 25° C.

In the same type of litter held under the same test conditions, the rate of decomposition is more or less variable. In order to ascertain the causes of this variation, the N, P_2O_5, CaO and SiO_2 content of the leaves was investigated. In some instances analyses of the most important organic constituents were made according to Waksman's method. On the whole, the rate of decomposition in the same kind of litter runs parallel with its N-content, and in most cases seems to be connected with it. Occasionally, however, other factors play a decisive part. It is interesting to note that variation in the phosphoric acid content of the leaves has not affected the rate of decomposition in the tests. The content of silicic acid has also been without influence.

The great variation within one and the same type of litter makes comparison between the different types of litter rather difficult. Nevertheless, the results go to show that at least two extreme groups may be distinguished, one with rapid and the other with slow decomposition. To the former group belong plants from mull forests, e.g., *Corylus Avellana, Fraxinus excelsior, Ulmus scabra, Rubus Idæus* and *Spiræa ulmaria* ; to the latter group belong the coniferous trees investigated, such as *Picea Abies* and *Pinus silvestris,* and typical raw humus plants, e.g., *Calluna vulgaris, Vaccinium Myrtillus, V. Vitis-idæa, Deschampsia flexuosa, Athyrium Filix-fœmina* and *Hylocomium parietinum.* Some species, e.g., *Fagus silvatica* and *Quercus Robur,* at times decompose relatively rapidly, at other times more slowly. The chemical causes for the different rate of decomposition of *various kinds* of litter seem to vary from one case to another, and no connection with the nitrogen content could be proved.

Certain exterior factors exercise a strongly modifying influence upon the rate of decomposition—in this respect the water and *p*H factors have been particularly investigated. The optimal hydrogen-ion concentration for the process concerned varies with different kinds of litter.

Certain differences in the rate of decomposition have also been

demonstrated in different kinds of litter, depending upon the water content.

Prof. B. Niklewski (Poznań). [Communicated by Dr. M. S. Niklewski (Rothamsted, England).]: *The Loss of Nitrogen in Manures and the Biological Activity of the Organic Fertilizers containing Humus.*

Liberation of free nitrogen in fermenting manure.—The cause of loss of nitrogen is the development of nitrifying bacteria in manure, e.g., *Nitrosomonas europæa* Winogr. and *Nitrobacter* Winogradsky. Their multiplication in manure is very abundant, and the nitrites and nitrates produced do not accumulate but are decomposed by the denitrifying bacteria to free nitrogen, which escapes into the air.

The nitrifying bacteria, particularly *Nitrosomonas*, are abundant even 150 cm. deep in cattle manure during its fermentation in the dung-yard. Evidently they are able to live when oxygen-access is very difficult.

The prevention of the liberation and loss of nitrogen may be effected by oversaturating manure with dung-water, since both fresh urine and fermented dung-water contain substances toxic to the nitrifying bacteria. Cattle urine is most toxic. Both nitrifying species are relatively resistant to the influence of temperature, since they were not killed in a manure which had been exposed to fermentation at 55–65° C.

The influence of manure and compost-humus upon the higher plants.—During the fermentation of organic fertilizers in the presence of air, water-soluble salts of humic acid are formed. These substances are subsequently precipitated by calcium, becoming inactive towards the higher plants. Whilst water-soluble, they stimulate plant growth.

Water-soluble humic substances constitute about 0·3 per cent. of fresh manure and 0·5 to 0·8 per cent. of manure exposed to a warm fermentation. A normal three-years'-old compost does not contain more than 0·01 per cent., and may be entirely free of water-soluble humus; it is called "Compost earth," and its influence upon the higher plants is very weak. A good "compost fertilizer" contains 0·5–0·10 per cent., and a compost prepared by biologic-dynamic methods contains 0·135 per cent. of such humus. Such a compost may be used efficiently in a quantity of 5 tons or less per hectare (ha).

In the following experiments three kinds of compost were used, having (1) 0·085, (2) 0·04 and (3) 0·02 per cent. of water-soluble humus. Doses of 4·25 kg., 2·00 kg., and 1 kg. of water-soluble humus were used per hectare.

In five series of experiments with each kind of compost the following increases of crop on an area of 25 sq. m. of field were obtained.

An increase of crop on 25 sq. m. in kg.

	Oats.		Barley.	
	Grain.	Straw.	Grain.	Straw.
Compost I	1·00	2·00	1·20	1·40
Compost II	0·50	1·20	0·70	0·90
Compost III	0·20	0·90	0·40	0·60
An inorganic fertilizer, equilant to the compost I ...	0·50	1·40	1·40	1·80

Calculated on a basis of the content of water-soluble humus in kg. to 1 are [100 sq. m.]:

Humus in grams. to 1 are in:	Oats.		Barley.	
	Grain.	Straw.	Grain.	Straw.
Compost I 42·5 g. ...	4 kg.	8 kg.	4·8 kg.	5·6 kg.
Compost II 20·0 g. ...	2 kg.	4·8 kg.	2·8 kg.	3·6 kg.
Compost III 10·0 g. ...	0·8 kg.	3·6 kg.	1·6 kg.	2·4 kg.

The influence of compost in the field depends upon the action of colloids and humic substances.

(*a*) *The Influence of humic substances upon the growth of roots.*—Products of fermentation inhibit markedly the growth of roots. A solution of humic colloids free of these toxic products of fermentation stimulates it. After sterilization at 130° C., manure extract showed the same stimulating effect.

(*b*) *Influence of humic substances upon the permeability of cell-plasma.*—Colloidal substances in manure extract act favourably upon the uptake of mineral salts by plants, facilitating the uptake of kations much more strongly than that of anions. The following average mineral uptake is calculated for 1 g. of dry substance of the roots.

	Absorbtion of $CaCl_2$.	
	$Ca^{\cdot\cdot}$	Cl'
In the absence of manure-extract	4,5 mg.	6,1 mg.
In the presence of manure-extract	7,5 mg.	7,9 mg.

	Absorbtion of $MgCl_2$.	
	$Mg^{\cdot\cdot}$	Cl'
In the absence of manure-extract	5,5 mg.	11,2 mg.
In the presence of manure-extract	8,3 mg.	10,5 mg.

It may be concluded that under the influence of those substances the structure of cell-plasma becomes changed, and consequently an absorbtion of ions becomes intensified.

(*c*) *Humic substances cause a chemotropic reaction of roots.*—Manure extract, free of the products of fermentation, exerts a chemotropic reaction which attracts roots. Even extract diluted one million times causes this reaction.

Dr. M. C. Rayner (London): *Remarkable Effects on Tree Growth obtained by the Use of Organic Composts and attributable to Microbiological Action.*

On certain soils it has been observed that seedlings of pine and other conifers suffer high mortality and severe inhibition of growth; root growth and mycorrhizal activity are markedly defective, although there is evidence of the presence of appropriate mycorrhizal fungi. The view was reached that the prevailing poverty in nutrients was not solely effective as a limiting factor to growth, but that other factors directly inimical to root growth were operative in certain cases.

A general hypothesis that qualitative changes in the humus lead to the production of a substrate unfavourable alike to root activities and to growth of the mycorrhiza-formers, led to the use of organic composts as a means of bringing about change of direction of the biological activities responsible for humus decomposition.

The effects on growth of the trees were remarkable in their uniformity, magnitude and persistence throughout successive growing seasons. They are illustrated by photographs of the plots and pot cultures, graphs recording average heights and height-frequencies of the populations of plots, and tables of dry weight values of corresponding pot cultures.

The conclusion is reached that their use as described, by modifying the course of humus decomposition, brings about qualitative changes in the organic constituents of a soil deleterious to tree growth, whereby the resultant products become markedly beneficent to the nutrition, growth and mycorrhizal development of young conifers. Evidence is presented that the action of the composts differs essentially from that of manures containing significant additions of available nutrients.

In the broad sense, the composts used are organic manures containing appreciable amounts of nitrogen, potash, and phosphates, the range of variation corresponding with that in the raw materials and other fluctuating causes. Agreement in results of successive annual experiments is not affected by such variations.

Assuming correct interpretation, there is, over and above this ordinary manurial action, one more lasting and significant in relation to tree nutrition, depending on modification of the intensity and course of the biological activities responsible for humus decomposition. There result qualitative changes in the organic constituents leading to the formation of a substrate favourable to root growth, mycorrhiza-development and general nutrition in the young trees. The further possibility of direct action by growth-promoting substances requires investigation.

This hypothesis is bound up indirectly with the mycotrophic habit in pines and other conifers, free mycorrhiza-formation being regarded as an index of favourable rooting conditions. The effects observed come into touch with Melin's researches on litter decomposition, likewise with recent observations on the growth-promoting activities of certain water-soluble organic substances.

If podsolisation in certain types of soil is bound up with the course taken during humus decomposition, there can be little doubt that the biological activities responsible for the latter may be influenced directly by qualitative changes in the organic substrate.

JOINT DISCUSSION *with* SUB-SECTION 3 *on* THE MICROBIOLOGY OF ENSILAGE PRODUCTION

Chairman: Prof. C. Stapp (Berlin).

Prof. A. I. Virtanen (Helsinki) **Opener.**

There is a great variety of biological processes in silage. In the freshly-cut crop, the respiration of the plant cells causes the combustion of soluble carbohydrates. At the same time, there commences a protein breakdown which, during the very early stages, is caused chiefly by the enzymes contained in the plant cells. The multiplication of micro-organisms commences immediately after ensiling. The initial flora contains a fair proportion of bacteria belonging to the coli-aerogenes group, as well as gelatine-liquefying organisms. These ammonifying bacteria readily destroy certain amino-acids, e.g., tryptophan. In the fresh material, lactic acid bacteria are comparatively few, but they multiply very rapidly during storage, so that a powerful lactic fermentation is already taking place on the second day. If the silage is made from material poor in protein, but with a high carbohydrate content, and is packed tightly, its acidity rises comparatively rapidly, whereby the

vegetative cells of the gelatine-liquefying organisms are first killed and, at about *p*H 4·5, even the coli-bacteria. Should sufficient sugar still remain, the lactic acid bacteria will be able to raise the acidity of the silage up to *p*H 3·6.

If high-protein crops, particularly legumes, are ensiled, large amounts of ammonia are formed already during the early stages. The lactic acid is thereby neutralized and the acidity of the mass rises but slowly, whilst the detrimental micro-organisms are not destroyed. Under such conditions, the spores of butyric acid bacilli will germinate and will then ferment the lactic acid present, with the formation of butyric and acetic acids, etc. Further acetic acid is formed on the fermentation of sugar by the coli-aerogenes bacteria. In addition to the foregoing decomposition of carbohydrates, the amino group of certain amino-acids is split off in the form of ammonia. The final product is therefore unsatisfactory, both as regards its physiological value and bacteriological properties. Tryptophan is either completely destroyed or present only in traces. The total nutritive losses are naturally very high.

In 1925–1928 the author devised a chemical process for the preservation of forage. This process is known as the A.I.V. method, its chief feature being the acidification of the fresh material with strong mineral acids to between *p*H 4 and *p*H 3, whereby harmful micro-organisms are destroyed and the formation of ammonia and the respiration of plant-cells are prevented. The A.I.V. method does not, however, involve a sterilization of the forage. This can only be accomplished by using very large quantities of acid, and the silage then becomes unfit for feeding. A *p*H-range of *p*H 4-3 does not yet require the presence of free mineral acids. If sufficient hydrochloric or sulphuric acid is added to the fresh material to bring the acidity of the mass, after completed reaction of the plant juices with the acid, to, for instance, *p*H 3·6, free mineral acid disappears rapidly, combining with the cations present. This results in the liberation of organic compounds of acid nature which are then responsible for the acidity of the mass.

The activity of lactic acid bacteria is not completely inhibited in A.I.V. silage, since *Lactobacillus pentoaceticus* in particular is still active below *p*H 4. Thus lactic acid is regularly formed, though slowly, in A.I.V. silage. At the same time, some acetic acid is also formed, evidently from pentoses, which are fermented by *Lactobacillus pentoaceticus* with the formation of lactic and acetic acids in the ratio of 6 : 4. The *p*H of the silage is, however, hardly affected by the formation of these weak acids.

Analyses of the micro-flora in samples of A.I.V. silage show that the chief organism present is a long rod (*Lactobacillus*), which forms lactic acid from hexoses and pentoses. There are also regularly found bacteria of the genus *Betacoccus*, which likewise ferment pentoses. Furthermore, spores of *B. subtilis*, *B. mesentericus* and *Amylobacter* are generally present. Yeasts are also represented

in A.I.V. silage, and are evidently responsible for the small quantity of ethyl alcohol (generally about 0·3 per cent.) found in this material.

The critical *p*H-values for the spores of butyric acid bacilli and for the lactic acid bacteria lie within the *p*H-range of the A.I.V. method (*p*H 4-3), and it might therefore be expected that a silage with a *p*H of, say, 3·3 would be different microbiologically from a silage of *p*H 3·8. In practice, however, this is not the case. Our extensive analytical material shows that lactic acid is found in A.I.V.—silage even with a *p*H of 3·3. Spores of butyric acid bacilli may also be found in A.I.V. silage of such acidity. This is apparently ascribable to the fact that the silage mass is a heterogeneous system, containing also soil particles, etc.

As was mentioned, *p*H 4 is the critical value of acidity in silage. Should the *p*H of the material be even slightly above 4, the detrimental processes, above all the formation of ammonia, will not be arrested. The formation of ammonia is, in fact, the best criterion for the quality of silage, so that it is possible to obtain a fairly accurate estimate of any silage by determining its ammonia content (total N basis). In an A.I.V. silage of *p*H 3·5—3·8, the formation of ammonia is almost negligible, only about 1 to 3 per cent. of the total N (after subtraction of the corresponding value for the fresh crop). Even this spurious amount of ammonia is derived from amides. On the other hand, in ordinary silage with its much higher ammonia content, only a small part of the ammonia originates from amides, most of it being derived from amino-acids through cleavage of the amino group. The fact that in the formation of ammonia certain important amino-acids are chiefly concerned, while others are less seriously affected, should be duly considered in determining the physiological value of silage.

Prof. R. Burri (Liebefeld, Switzerland) [Communicated by Dr. C. Stapp (Berlin)] : *Die Mikrobiologie verschiedener in der Schweiz gebräuchlicher Silofutter-Typen.*

Vor bald 20 Jahren haben die an unserer Anstalt ausgeführten Arbeiten zur Abklärung der Ursachen der schädlichen Wirkung der Verwendung von Silofutter auf die Emmentalerkäserei den Beweis erbracht, dass der Gehalt solchen Futters an Sporen eines bestimmten Typus von Buttersäurebazillen auf verschiedenen Wegen eine Infektion der Käsereimilch und des Käses bewirken kann, in welchem sich dann eine Buttersäuregärung mit ihren schädlichen Folgen entwickelt. Die Tatsache, dass sich heute ein neuer Silotypus, der von Prof. Virtanen erfundene Mineralsäure-Silo, auch in der Schweiz auszubreiten beginnt, veranlasste uns, im Interesse der nationalen Emmentalerkäserei auch der Mikrobiologie dieses Silo-Typus unsere Aufmerksamkeit zu widmen, umsomehr, als die holländischen Forscher van Beynum und Pette in neuester Zeit sehr interessante Untersuchungen über die Natur der in Silofutter

auftretenden Buttersäurebazillen im allgemeinen und diejenigen des Mineralsäure-Silos im besondern veröffentlicht hatten.

Wir haben im Laufe des Winters 1935/36, 54 Proben von Silofutter bakteriologisch mit besonderer Berücksichtigung der Buttersäurebazillen untersucht. Von den Proben entfielen 33 auf das Mineralsäureverfahren, 21 auf andere Verfahren, in der Hauptsache solche, bei welchen im Gegensatz zum Mineralsäureverfahren eine Selbsterwärmung des Futters eintritt.

Zum Nachweis der Buttersäurebazillen bedienten wir uns wie schon seinerzeit der Dextroseagarkultur in hoher Schicht (sog. Schüttelkultur), die in verschiedenen Verdünnungen mit der pasteurisierten Silofutter-Emulsion beimpft war. Wir erhielten durch Buttersäuregärung hervorgerufene Gasbildung :

beim Mineralsäureverfahren in 26 Fällen, d.h. bei 72,7 prozent der Proben,

bei den übrigen Verfahren in 19 Fällen, d.h. bei 90,5 prozent der Proben.

Nach van Beynum und Pette bekommt man bei Verwendung von Dextrosenährböden nicht immer nur eine Art von Buttersäurebazillen, sondern nicht selten deren zwei. Die genannten Autoren unterscheiden ein *Clostridium saccharobutyricum*, welches aus Disacchariden und Alkoholen Buttersäure bildet, nicht aber aus Laktaten, und ein *Clostridium tyrobutyricum*, welches Laktate vergärt, nicht aber Disaccharide und Alkohole. Nur der letztgenannte Organismus wäre für die Käserei schädlich. Wir haben die Differenzierung bei der Mehrzahl unserer Buttersäurebazillenstämme durchgeführt und folgendes festgestellt :

unter 24 aus Mineralsäure-Silos isolierten Stämmen waren 20 laktatvergärende, d.h. 83,3 prozent,

unter 18 aus andern Silos isolierten Stämmen waren 4 laktatvergärende, d.h. 22,2 prozent.

Darin liegt eine Bestätigung der Angaben der holländischen Autoren, wonach nicht alle mittels Dextrosenährböden isolierten Stämme Laktatvergärer sind. Bemerkenswert ist aber ein von uns gemachter weiterer Befund, laut welchem 6 der untersuchten Stämme sowohl Dissacharide und Alkohole als auch Laktate vergärten und somit die Gärungsfunktionen von *Cl. saccharobutyricum* und *Cl. tyrobutyricum* in sich vereinigen. Logischerweise müsste man also neben den beiden genannten eine dritte Art aufstellen. Wir vermuten aber, dass die drei verschiedenen Aeusserungen des Gärungsvermögens nicht so konstanter Natur sind, dass sie als Klassifizierungsprinzip benützt werden können.

Prof. C. Gorini** (Milan) : ***Ensilage italien et ensilage finlandais.

Les ensilages butyriques sont dangereux et les ensilages lactiques sont bienfaisants aux animaux domestiques. La fermentation lactique peut-être obtenue rapidement par deux moyens : l'en-

semencement de ferments lactiques et la discipline de la température entre 40°–50° C.

Même l'ensilage finlandais (A.I.V.) s'avantage de la fermentation lactique. En effet, cet ensilage doit rejoindre aussitôt que possible un *p*H entre 3 et 4 pour empêcher les ferments butyriques sans être nuisible aux vaches. Cette acidité n'est pas à cause des acides minéraux ajoutés qui forment des sels avec les constituants basiques du fourrage, mais aux substances acides organiques qui sont libérés et, surtout, à la fermentation lactique. En Italie l'ensilage A.I.V. est fabriqué par l'addition des acides minéraux. On y trouve souvent des ferments butyriques ; peut-être, parcequ'il est difficile de doser les acides minéraux selon la qualité du fourrage et du sol, et de les distribuer uniformément dans la masse, surtout si les couches ne sont pas assez minces.

Ces causes d'erreur sont écartées par l'ensemencement des ferments lactiques, qui se propagent plus indépendamment de la qualité et de l'épaisseur du fourrage, surtout si on les emploie dans une solution sucrée.

Il y aurait peut-être avantage à associer le système italien au système finlandais ; on rejoindrait plus rapidement et plus uniformément le *p*H nécessaire et il n'y aurait pas formation de lactates qui favorisent certains types de ferments butyriques.

Dr. L. A. Allen (Reading) : *The Part played by Bacteria in the Fermentation of Grass Silage.*

Experiments have been carried out to discover the types of bacteria developing in grass silage under normal conditions during the period of ripening—particularly when young grass of a high protein content was used as the crop.

On fresh grass there are usually between 100,000 and 1,000,000 coliform bacteria and between 1,000,000 and 10,000,000 lactobacilli per gram and both these groups of bacteria are found in large numbers in the early stages of ripening of the silage. The predominant coliform (to which the name *B. aerogenes graminis* has been given) has been found, however, to be a type which grows well at 20° C. and 30° C. but is unable to grow at 37° C., so that it is quickly rendered inactive by the rising temperature of the silo contents.

The lactobacilli persist in large numbers for several weeks and are regarded as mainly responsible for the development of lactic acid. They are mainly of the *Streptobacterium plantarum* type, forming lactic acid (usually inactive) and small quantities of acetic acid. There are only 10–100 spore-forming anaerobes per gram of fresh grass and they do not multiply actively in silage until several days after ensiling, when there are suitable anaerobic conditions. They may then attain numbers between 1,000,000 and 100,000,000 per gram. *Clostridium sporogenes* was found in all samples of silage.

The effect was studied of adding various substances, such as dried whey, liquid whey, or molasses, with and without cultures, to the grass at the time of ensiling. It was concluded that the addition of soluble carbohydrates encouraged the growth of lactobacilli and the production of acid in the silage, thereby tending to restrict the growth of anaerobes in the later stages. Molasses appears to be a suitable form of carbohydrate for this purpose. The growth of the lactobacilli may also be encouraged still further by incorporating a mixed culture when the grass is ensiled.

Prof. G. Ruschmann (Landsberg a/Warthe), hebt die Bedeutung des Zuckers für die Grünfuttereinsäuerung hervor der lange Zeit als einziges bemerkenswertes Zusatzmittel betrachtet wurde. Dann kamen die verschiedensten Mineralsäuren in Gebrauch, durch die man eine Herabsetzung des *p*H-Wertes im Grünfutter von vornherein erreichte. Auf diese Wiese wird die Buttersäuregärung, die bereits durch den Zuckerzusatz in den meisten Fällen unterbunden war, unmöglich gemacht. Doch ist es nicht richtig, die Güte eines Sauerfutters einseitig oder vorwiegend nach seinem Buttersäureghalt zu beurteilen. Auch ein buttersäurehaltiges Futter hat noch seinen Wert, wenn auch seine Verwendung in bestimmten Fällen ausgeschlossen sein sollte.

Prof. R. E. Buchanan (Ames, U.S.A.), asked Dr. Virtanen what differences were to be found in different legumes and other ensiled crops as to their buffer effect in Virtanen (A.I.V.) silage production ; also, were decided differences to be found in the buffer or neutralizing values of crops grown in soils of differing acidity.

Prof. A. I. Virtanen (Helsinki).

Die Behauptung von Prof. Dr. Ruschmann, dass die Buttersäuregärung im Futter gar nicht so schädlich wäre und die Qualität des Futters herabsetze, wie man das früher ganz allgemein angenommen hat, ist m.E. nicht zutreffend. Zwar sind kleine Mengen Buttersäure den Tieren wahrscheinlich gar nicht schädlich, aber das Vorkommen der Buttersäure im Futter ist ein Beweis dafür, dass die Buttersäuregärung im Futter durch schnelle Aziditätssteigerung nicht gehemmt worden ist und dass infolgedessen auch die anderen schädlichen Spaltungsprozesse wie Proteolyse im grossen Massstabe erfolgt haben. Die Qualität eines solchen Futters ist nicht gut und die Verluste bei der Konservierung sind gross.

In reply to Prof. Buchanan : Different plants require different quantities of acid to bring about the same acidity in the silage. Alfalfa requires more acid than other plants. Non-leguminous plants generally require considerably less acid than the leguminous

species. Thus, for instance, in Finland we use 14–15 l. of 2 N acid (H_2SO_4 or HCl) per each 200 kgs. red clover, for timothy 12 l. and for marrow-stem kale 8 l. In general it can be said that crops with a higher protein content require more acid than low-protein crops in order to produce the same *p*H-value.

The type of soil influences largely the composition and content of mineral matter in plants and also its neutralizing value. For instance, when red clover has grown on a soil with *p*H 5·0–5·5, 12 l. of 2 N acid per 200 kgs. of the material suffice to bring its acidity to below *p*H 4, while 14–15 l. are required for 200 kgs. of red clover grown at *p*H 6·5, and up to 16–17 l. for a crop from a soil with *p*H 7·5, provided, of course, that the dry matter content of the material is the same in each case.

DISCUSSION *on* THE DESTRUCTION AND PROTECTION OF WOODS AND CELLULOSIC MATERIALS

Chairman : Prof. R. E. Buchanan (Ames, U.S.A.).

Dr. A. C. Thaysen (Teddington, England), **Opener** : *The Biological Destruction of Cellulose and its Prevention.*

The writer refers to certain aspects of the association of micro-organisms with the natural elimination of cellulose, and expresses the view that part of our knowledge on this subject requires reconsideration. He maintains that the validity of the work which has been done may in some cases be questioned because writers have failed to give a clear definition of the term " destruction " of cellulose. He draws attention to the inadvisability of attempting to classify the known cellulose-decomposing micro-organisms before they have been studied in their true morphological relationship, and refers briefly to the suitability of the methods which have been adopted in identifying micro-organisms as agents responsible for the natural elimination of cellulose.

The writer proceeds to discuss two further aspects of the microbiological decomposition of cellulose, (1) the participation of micro-organisms in the digestion of cellulose by herbivorous animals, and (2) the means which are available, and those which for preference should be adopted, for the prevention of the biological destruction of cellulose.

With regard to the former aspect, the writer claims that the mere isolation of cellulose-decomposing micro-organisms from the intestinal content of an animal is no proof that it has taken a symbiotic part with the host in the digestion of cellulose. Reference is made to recent work which indicates that, among the intestinal microflora, the protozoa at least appear to be merely commensals.

On the subject of the protection of cellulose against microbiological destruction the writer recalls that the methods which

have been adopted for protection of textiles have been based on the assumption that germicidal substances added to the textile would ensure protection. He proceeds to show the fallacy of this assumption and comes to the conclusion that the only two reliable ways of securing protection are, (1) either so to modify the individual unit of the cellulosic material, the cellulose molecule, that it is no longer affected by the enzymatic activity, by which it must be assumed that the attacking micro-organisms are enabled to decompose it, or (2) by modifying the activity of these enzymes.

***Prof. H. Pringsheim** (Geneva) : *Fermentative Degradation of Wood-compounds.*

Wood destruction is largely caused by micro-organisms. Wood mostly consisting of cellulose, hemicelluloses (xylan), and lignin, is protected by the latter, the more resistant part. The capillary structure of cellulose is reduced by incrustation ; Schweitzer's reagent dissolves cellulose, but hardly affects wood. Micro-organisms react on cellulose only when they are in direct contact with it. Under anaerobic conditions, such as under water, lignin, and consequently wood, is particularly resistant ; wood, however, is more easily attacked by mycelia-fungi than by bacteria. The latter react in nature more on straw, e.g., manure.

Ferments for lignin are as yet unknown. True cellulose has been discovered (Pringsheim, 1912) in bacteria and fungi, especially in thermophilic bacteria. By partial poisoning with iodine, sugar-splitting enzymes are destroyed, while hydrolysing enzymes remain. Thus glucose and cellobiose are found as intermediary products. Cellobiose as enzymatic product from cellulose is important for constitution of the latter, built up according to cellobiose principle. The theory that intermediary sugars and not end-products, viz., fatty acids, are used by the animal body, especially by ruminants, is now generally accepted.

Karrer found that the cellulase of helix was especially active, and obtained with it complete solution of hydrated cellulose. His studies on surface influence with different kinds of rays are important.

There is an interesting specific difference among the ferments reacting on wood-components. Cellulose built up from glucose is split by a ferment other than xylan, which is built up from xylose. Cellobiose β-glucosidic enzyme is different from cellulase in spite of the same building principle of the substrate, as may be proved by the separation of cellobiose from lichenase (Pringsheim-Beiser, 1926), lichenin being a cello-polyose of shorter chain-length. Grassmann-Zechmeister, with Aspergillus ferment, found oligosaccharase was active on cellobiose, -triose, -tetraose and -hexaose, but not on cellodextrin (mol. 1500), while polysaccharase was active on cellulose, dextrin and hexaose. Consequently the chain-length is decisive for ferment action.

Mr. W. G. Campbell (Princes Risborough, England): *The Chemical Reactions induced by Wood-destroying Fungi.*

Chemical analysis was first applied to the study of wood decay in 1917 by Rose and Lisse (Ind. Eng. Chem. 1917, 9, 284). This early work has been extended so that at the present time a considerable amount of information exists concerning the action of fungi on wood substance. In considering the higher fungi, which are recognized as destroyers of converted timber, it is suggested that one of the most profitable methods of approaching the subject of wood preservation resolves itself into two main branches, namely :—

(1) The characterization of the chemical reactions which take place during decay, and
(2) The study of ways and means to treat wood so that the key reactions are inhibited.

The author deals principally with the first of these two branches.

Evidence, obtained from a variety of sources, including work carried out by the author, indicates that fungi of the brown rot type attack chiefly carbohydrates, and decomposition of lignin is comparatively slight in extent. The chief type of enzymic reaction which takes place in brown rot of wood is hydrolysis.

The chemistry of the white rots of wood is more complicated. All the more recent chemical evidence indicates that fungi which produce this type of rot decompose carbohydrates as well as lignin, but that there is an absence of uniformity with regard to the order and extent of attack on the various wood components. White rots of wood can be subdivided on chemical grounds into three groups as follows :—

(1) Those in which lignin and hemicelluloses are attacked in the early stages and in which the incidence of attack on cellulose is delayed.
(2) Those in which cellulose and its associated hemicelluloses are attacked in the early stages and in which attack on lignin and the remaining hemicelluloses is delayed.
(3) Those in the early stages of which carbohydrates and lignin are attacked simultaneously but in varying proportions.

The chief enzymic reactions which are effective in white rots are oxidation and hydrolysis.

Mr. W. P. K. Findlay (Princes Risborough, England) [Communicated by Mr. K. St. G. Cartwright (Princes Risborough)]: *The Protection of Wood by Antiseptics.*

Though the art of wood preservation is quite an old one, the principles involved have been little understood. By empirical and practical tests on a large number of chemicals, certain substances, particularly the oils distilled from coal tar, were found to be effective. The efficiency of a preservative depends partly on its toxicity to fungi, and research on preservatives was long hampered

by the lack of a suitable laboratory test for toxicity. A standard laboratory test for the toxicity of wood preservatives, which was agreed to by an international conference at Berlin in 1930, has now been published. It is emphasized that the value of any preservative must be considered in relation to the material on which it is to be used—for instance, a water soluble preservative may be most effective under cover but quite unsuitable for use in the open. There is no one best preservative for all purposes—for some purposes dark colour and oily nature may be advantageous, while for other purposes they may prevent the use of the preservative entirely. Future progress in wood preservation will depend on a more exact knowledge of the actual chemical processes involved in fungal decay and on the use of standardized methods for testing the relative efficiency of antiseptics. It is necessary to know very much more about the exact mechanism by which wood preservatives act on fungi and how their toxic efficiency is related to their chemical composition.

Dr. S. Śnieszko (Cracow): *New Method of isolating Anaerobic Thermophilic Cellulose-fermenting Bacteria.*

The new method of isolating thermophilic cellulose-fermenting bacteria is in principle similar to my former one, being based on the diminishing of the quantities of the associated bacteria in relation to the specific organism. Isolation is effected as follows: Having obtained a strongly fermenting culture of the cellulose bacteria, it is purified on cellulose agar Petri dishes according to the method of Tetrault. Having thus obtained the purified culture at the peak of fermentation, it is thoroughly mixed and then 1 c. cm. is inoculated into broth in a 100 c. cm. Erlenmeyer flask. The flask is closed with a rubber stopper through which glass tubes are passed permitting the bubbling-through of sterile air. The flask is incubated for four days at a temperature of 60-65° C. During this period samples are taken from the flask every six hours and heated for 10 minutes at 100° C. Immediately after each heating, dilutions are prepared from each sample which are inoculated into tubes of cellulose broth containing a yeast suspension. The cellulose broth with the yeast suspension is prepared as previously described.

The aeration of the broth culture ensures rapid germination of the aerobic and facultatively anaerobic contaminating organisms, and prevents the germination of the spores of obligate anaerobic cellulose-decomposing plectridia. In this way it is possible to kill off a large number of contaminating organisms and to obtain a mixture in which the spores of the cellulose organism are in a large majority.

Dr. E. J. Butler (London).

Attention may be called to the recent advances in the understanding of the parasitic action of fungi, due to the work of Prof.

W. Brown and his colleagues on the enzymic theory of parasitism. Rather similar factors may be concerned in timber decay, and this problem may be attacked by working with the enzymes rather than with the fungus, and by using a simpler and better-known type of fibre than the woody fibre of trees, e.g., the cotton fibre. Progress from the simple to the complex would naturally follow.

Mr. K. St. G. Cartwright (Princes Risborough).

With reference to Dr. Butler's remarks concerning the destructive action of fungi on the cementing substance of the cell wall, I am collaborating with Dr. Astbury, of Leeds University, in an examination of the possibility of throwing light on this aspect by means of X-ray analysis. Sections of Sitka spruce wood are analysed after varying exposure to the enzymes of wood-destroying fungi, some of such sections being exposed to mechanical tests before analysis.

Mr. W. G. Campbell (Princes Risborough), thanked Dr. Butler for his suggestion and said that it was his ambition to be able to isolate the individual enzymes of wood-rotting fungi and to investigate their effect on the individual wood components. The problem was, however, difficult, as it was, as yet, impossible to fractionate wood into its components without changing their constitution. Furthermore, it was difficult to persuade wood-destroying fungi to grow well enough on artificial media to permit of good yields of enzymes.

Dr. E. Walker (Oxford).

Cytophaga should be considered as a true cellulose decomposer, inasmuch as the cellulose is converted into a soluble form and thus brought within the sphere of attack by other micro-organisms.

Winogradsky's creation of a new class for *Cytophaga* is based upon a biochemical differentiation, viz., the formation of mucilage.

The question was raised as to the nature of the initial reaction in the attack on cellulose.

Dr. J. Pochon (Paris).

Les bactéries du tube digestif, au point de vue cellulolytique, peuvent être classées en deux grands groupes physiologiques. D'une part des bactéries présentant tous les caractères biologiques des bactéries cellulolytiques du sol. D'autre part des bactéries cellulolytiques à caractères bien particuliers, ne pouvant être cultivées que sur un milieu complexe possédant tous les éléments du contenu intestinal de l'animal hôte, e.g. *Plectridium cellulolyticum.* Cette spécificité nécessaire du milieu de culture est un premier argument en faveur de leur rôle dans la digestion. Il en est un second—l'identité du métabolisme de la fermentation dans la panse et de celle

obtenue *in vitro* avec une culture pure. Il en est une troisième—l'explication qu'apporte, dans la nutrition des Ruminants, la transformation de la cellulose en glucose, en milieu acide, par *Pl. cellulolyticum.* Enfin toute une série de faits tendent à prouver que ces bactéries à caractères biologiques spéciaux ne sont que les bacilles cellulolytiques du sol secondairement adaptées au milieu intestinal. Cette adaptation n'est-elle pas le témoin d'un rôle vrai dans la digestion ?

Tous ces arguments n'apportent, il est vrai, qu'une probabilité. Il est, de plus, à expliquer la lenteur de la cellulolyse *in vitro* par opposition avec sa rapidité *in vivo.*

Dr. A. C. Thaysen (Teddington), in reply to Dr. Pochon asserted that the term " symbiotic complex," if used too freely to explain the rapid destruction of cellulose in the intestines of ruminants, became merely a cloak for ignorance. The intestine of ruminants must possess some unknown factor which enabled cellulose breakdown to proceed so much more rapidly than could be effected by biological means.

The speaker was opposed to the incorporation of all cellulose-splitting organisms into a watertight class, because some, at least, would of necessity fall outside the class, e.g., cellulose-splitting *Myxobacteria.*

Dr. A. G. Norman (Rothamsted).

Dr. Thaysen very properly raised the question of the criteria to be applied in ascribing cellulose-decomposing ability to any organism. Some micro-organisms, particularly the lower fungi, do not grow, or make only very scanty growth, when supplied with cellulose as the sole carbohydrate source ; yet, when inoculated on to sterilized plant materials such as straw, they bring about an extensive decomposition, largely accounted for by removal of cellulose. As an instance of this among bacteria, *Mycobacterium agreste*, not known to be a cellulose decomposer, is capable of removing more cellulose from straw than the well-known cellulose decomposer *Cytophaga hutchinsoni.* The fact that wood celluloses and plant celluloses are chemically different from pure cotton or purified filter-paper has not been sufficiently taken into account in work on cellulose decomposition. The fact that there may be xylan or other carbohydrates so intimately associated with the true cellulose molecules as to be an integral part of the cellulose framework may alter the extent of the attack which organisms are able to effect.

The question of the extent of interference in analytical methods from the presence of synthesized microbial tissue in decomposed materials was raised. In distinction from wood, it should be said that with materials such as straw, or plant residues used in composts, the amount of synthesized tissue which may result in complete

decomposition forms a considerable proportion of the final weight, from 15 to 25 per cent. not being uncommon. It has been established that this provides no serious errors in the determination of cellulose or pentose units, but, owing to the protein content, may cause interference in the determination of lignin, a source of error which at present it is not possible wholly to overcome.

Dr. S. Śnieszko (Cracow).

Glucose and other sugars may be used in place of cellulose by some of the pure cultures of aerobic cellulose decomposing bacteria of the Kellerman type.

DISCUSSION *on* PROBLEMS OF BIOCHEMICAL PURIFICATION OF SEWAGE AND OF TRADE EFFLUENTS

Chairman : Dr. A. Parker (London).

Dr. H. Heukelekian (New Brunswick, U.S.A.), **Opener** [Communicated] : *The Contribution of Microbiology to the Problems of the Biochemical Purification of Sewage.*

The general trend of investigation in the biochemistry of sewage purification has been as follows : (1) The enumeration of the numbers and groups of micro-organisms in the different treatment processes, (2) the gross and specific transformations brought about by micobiological activities.

The following is a brief discussion of some of the pertinent problems in the biochemistry of sewage treatment.

1. *Nitrification.* Nitrification in aerobic biochemical sewage treatment processes is a fundamental and necessary accompaniment of certain transformations, but is not an aim in itself. A few successful isolations of the nitrifying bacteria from activated sludge and trickling filters have been made. Nitrification in these processes appears only after the sewage has been subjected to preliminary purification and oxidation. It is not necessary to postulate an adsorption and storage of ammonia by the biologically active film prior to oxidation to nitrates simply because bacterial oxidations cannot proceed rapidly enough. Adsorption of ammonia by the nitrifying organisms within the biologically active film is necessary, and may be the only kind of adsorption taking place.

2. *Transformation of Sulphur Compounds.*—Organic and inorganic sulphur compounds are a potential source of odours in sewage treatment processes. The reduction of sulphates to hydrogen sulphide in sewers is the most important phase of this problem. Sewage readily becomes devoid of oxygen due to the difficulty with which oxygen goes into solution and the high oxygen consuming capacity of the impurities. In the presence of organic compounds in sewage which can serve as hydrogen donators, the sulphate

becomes reduced to hydrogen sulphide, and the organic compounds become oxidized. The number of species of bacteria capable of reducing the sulphates is comparatively few. The reaction is hastened at summer temperatures and by the presence of the organisms in the slime collected in the sewers. The oxidation of the hydrogen sulphide in the sewer atmosphere results in the production of sulphuric acid and considerable damage to the sewer pipes. Chlorination to destroy the sulphate-reducing organisms has been found to be the best remedy.

3. *Clarification of sewage.* The mechanism of clarification of sewage has been attributed to physical—chemical and micro-biological reactions.

The clarification of sewage in aerobic processes, such as in activated sludge and trickling filters, is of such rapid nature that it resembles the rate of physical reactions such as adsorption and base exchange. It has been assumed that biological reactions could not proceed rapidly enough to be responsible for it. Predominant microbial activity is supposed to take place after the initial removal of materials from suspension and solution, and their transference to the solid phase. Micro-organisms oxidize the adsorbed impurities and make possible the regeneration of the adsorptive capacity of the floc. The biologically-active film has therefore two functions, in the clarification stage it serves as an adsorbing surface and in the oxidation stage it serves as an inoculating agent. It is however to be expected that the rate of oxidation is greatest during the initial period coincident with the period of clarification and there is no reason for a lag of microbial activity during this period.

4. *Transformation of organic matter.* In all the different phases of biochemical sewage treatment processes, both of aerobic and anaerobic types, transformations of organic matter constitutes the most important problem. In aerobic processes, such as activated sludge and trickling filters, the primary objective is the rapid purification of the liquid phase and not the complete stabilization and oxidation of the solids. But even here the type and degree of oxidation of organic matter of the biologically active slime has an important bearing on the purification of the liquid phase. The complete aerobic decomposition of sewage solids has not been utilized on a practical scale as a means of biochemical stabilization of the putrescible solids. The efficiency of the aerobic processes is higher than the anaerobic digestion, but it is not economical to maintain aerobic conditions in the sludge with a high moisture content.

The anaerobic decomposition therefore remains the established and valuable means of stabilizing the sewage solids prior to their disposal. The C-N ratio of the raw solids is approximately 10 : 1, so that nitrogen seldom becomes a limiting factor and ammonia nitrogen accumulates in the course of digestion. The organic acids produced from the anaerobic digestion of the raw solids reduce the *p*H

to such an extent that subsequent bacterial activity is retarded for a long period. The slow utilization of these acids by bacteria and their neutralization by ammonia from the slow proteolysis, increases the *p*H value and in consequence the rate of decomposition. Lime accelerates the rate of decomposition by neutralizing the accumulated organic acids. Normally the acid digestion phase is prevented by mixing the raw solids with a suitable portion of digested sludge, and the digestion is completed within a shorter time. Ripe sludge has a dual rôle as a buffering agent and as a mass inoculum. Little is known regarding the specific organisms in the ripe sludge. It has been shown that there is a succession of groups of organisms corresponding to the different stages of the breakdown of organic matter.

Of the specific components of sewage solids the ether soluble fraction is the most abundant, and in spite of general conception it is readily decomposed under anaerobic conditions. Profound changes in the organic nitrogen component also take place in the anaerobic stabilization of sewage solids. Ammonia nitrogen produced from proteolysis accumulates since nitrification is precluded. Direct liberation of nitrogen gas from the nitrogenous compounds is not probable.

The gas produced consists mainly of CO_2 and CH_4 which is used as an index of the rate and stage of decomposition. The course of cumulative gas production is an S-shaped curve. The higher the fat content of the raw solids the greater is the yield of gas and the higher the percentage of methane.

The theory that in anaerobic dissimulation process water may act as an oxidizing and reducing agent has been applied to the anaerobic digestion of sewage solids.

The well-known effect of temperature on biological reaction has been found to apply to sludge digestion. Four distinct zones of activity on the basis of temperature have been suggested (1) Cryophilic below 10° C.; (2) temperate zone 10°–28°; (3) Intermediate zone 28°–42° C.; and (4) thermophilic zone above 42° C. In the thermophilic and temperate zone the digestion proceeds according to ordinary chemical law, the time decreasing and the rate increasing as demanded by the law of Arrhenius. The type and course of decomposition are not materially changed by the temperature. Practical applications of the acceleration of digestion by the temperature has been made by the use of heated tanks. Temperatures as high as 30°–35° C. have been maintained under practical operating conditions.

The phenomenon of thermogenesis in the aerobic decomposition of carbonaceous materials has led to speculation regarding the liberation of heat in anaerobic digestion of sewage solids. Owing to the high moisture content of sewage solids this factor, even if operative, is not effective in raising the temperature of the digesting mixtures appreciably.

Prof. Jan Smit (Amsterdam): *Bulking of Activated Sludge.*

The sludge disease known as " bulking " has been observed in all parts of the world, and numerous causes and many remedies have been advocated. The view generally held is that under-aeration creates a condition of overloading, which results in sickness.

The character of this disease has been subject to some controversy, but most of the workers agree that " bulking " consists of a change of composition and specific gravity, caused by the excessive development of *Sphærotilus*, accompanied by a decrease of the ash content, which are the causes of the bad settling.

Scott (1928) first contested the importance of adequate aeration, and suggested that certain constituents (milk waste, starch, brewery waste) were responsible. Many investigators have likewise claimed that sugars may be the cause. Investigations at Amsterdam, where heavy bulking occurred, led me to the view that sugars might be excluded in the case of ordinary domestic sewage, because its normal sugar content (less than 50 parts per million) is far too low, whilst added sugar is quickly decomposed.

Schmiedt has held the view, that low temperature of the blown-in air and H_2S are causes of bulking. My own investigations have not confirmed this view: 10 ppm. of H_2S did not stimulate *Sphærotilus*. Also, strong evidence against this view is found in the results of investigations in which the treatment of sewage was split into two stages. Bulking rapidly occurred in the activated sludge of the second stage, though there was no sugar or H_2S present. This difficulty has been avoided by the use of filter-beds which can be treated with 3 times the usual amount of first-stage effluent, bulking being thus avoided.

The means of preventing the disease are limited. One of the most effective seems to be the thorough re-aeration of the return sludge. This has the effect of diminishing the avidity of oxygen absorption (Nordell number). E. E. Smith (Lima, Ohio), advocates the addition of 1-2 ppm. of chlorine to prevent and to cure the disease.

Thus it would appear that the development of *Sphærotilus* may influence the overloading of sludge, although the exact relationship is not yet clear.

Mr. N. W. Barritt (Rothamsted, England): *The Mechanism of the Activated Sludge Process.*

Sewage purification consists of two processes :—

1. Oxidation of organic matter to CO_2, NH_3 or N_2O_5.
2. Removal of organic matter by precipitation, which may occur in two ways :
   (*a*) by the growth of organisms and their aggregation;
   (*b*) agglutination and precipitation of the organic matter.

The precise part played by physical and biological factors has not hitherto been clearly understood.

On the physical side, it is known that when sewage is merely

pumped from one tank to another it suffers an increase in settleable solids. Also, mechanical agitation of filtered sewage liquor or the bubbling of oxygen-free gases will, in about 6 hours, reduce by 50 per cent. the amount of organic carbon and nitrogen remaining in solution after centrifugation. No explanation of this supposedly physical effect has yet been found. It does not occur when the liquid is cooled below 10° C., but it continues at temperatures as high as 80° C.

On the biological side, Cook and Stephenson, using a washed suspension of *Bact. coli* in the Barcroft apparatus, obtained an oxygen uptake for a dextrose solution of 75 per cent. of the theoretical maximum in 4 hours' contact. The rate of oxygen uptake is obviously governed by the relative amounts of the oxidative mechanism, and oxygen supply in relation to the substrate. The suspension of bacteria was a dense one of the order of 12,000 million per c.c. Such a concentration of bacteria is never reached in sewage treatment.

In practice it would not be possible to work with such a high concentration of the oxidative mechanisms. The mixture must be capable of being manipulated so that the water can be readily separated from the organisms, which are then available for re-use. It is, however, unnecessary to work with a dense population of the organisms. Sewage liquors rarely reach a bacterial population of 1,000 million. Most of the flora occurs in the form of " flocs " of micrococci of varying sizes, and in the presence of protozoa the numbers of dispersed bacteria seldom reaches 200 millions per c.c., of which only a portion are viable. These flocs may contain fungi, *Actinomycetes*, as well as a mixed bacterial flora, and are capable of proliferation. Portions may become detached and give rise to new flocs.

Activated sludge may contain any or all of the organisms given in the following chart, and its properties depend upon the particular organisms which predominate. Obviously any organism which can live in the sewage medium will promote the removal of organic matter in proportion to its ability to utilize the material in oxidative and assimilatory processes.

Strength of sewage in relation to predominant organism.

B.O.D.	*Organism.*
140	
120	Fungi.
100	Yeasts.
80	Actinomycetes.
60	Bacteria (i.e. Rods, Spirochætes, Vibrios) Flagellates and Amœbæ.
40	Ciliates (i.e. *Paramæcium, Colpoda, Stylonychia, Chilodon, Gastrostyla*); and Eelworms.
20	Bacterial zooglœa (i.e., *Micrococcus*), and Ciliates (i.e. *Aspidisca, Epistylis, Vorticella, Carchesium*).
0	Rotifers.

It is improbable that any organisms exist having a metabolism of which the only excretory product is CO_2 except under very exceptional conditions. The nature of excretory products will be influenced to a large extent by the substrate. The higher the energy value of the substances utilized the more complex will be the substances excreted, and the more varied will be the population required to effect its complete oxidation.

Recent work at Rothamsted on the reduction of B.O.D. of various sewage liquors by activated sludge has confirmed that 80 per cent. of the B.O.D. of the liquid is removed during the first 4 hours, and that similar results are obtained whether the agitation occurs with or without aeration of the mixture. The rate at which it occurs depends upon the nature of the sludge, but it only occurs very slowly in the absence of sludge. It is also affected by temperature, the optimum, as determined by Richards and myself, being about 30° C.

These results are not exactly similar to those obtained by Williams without sludge, since the methods of manipulation are different. In these experiments the organic matter removed is of macroscopic size, the liquid being merely paper filtered before analysis. The fact that oxygen is not immediately necessary and that the reduction of B.O.D. occurs with such rapidity suggests a more purely physical explanation for the first part of the purification process.

Its dependence upon the nature of the sludge and also to some extent upon temperature, distinguishes it from purely physical adsorption at surfaces. A possible explanation is in terms of the "precipitin" reaction, the polluting substances being antigenic towards certain specific organisms.

There is some evidence to show that this reaction is favoured by the presence of certain ciliates and rotifers. Further work on this aspect is in progress to determine to what extent the indicator organisms take an active part in the process.

Dr. W. R. Wooldridge (London) : *Oxidations in Sewage Systems.*

The oxidation of constituents of sewage, as distinct from clarification processes, is important in most methods of sewage disposal. These oxidations depend largely on living organisms and their enzymes. In the ordinary five-day biochemical oxygen demand test of sewage effluents no dissolved oxygen is absorbed by the sewage if the system be sterile ; the addition of washed bacterial cells or of "dead" cells containing certain active enzymes will effect the oxidation of sterile sewage under the conditions of this test. Sewage sludge, but particularly activated sludge, possesses many oxidation-reduction enzymes. Using a Barcroft micro-respirometer, it has been shown that sewage or a sewage-sludge mixture rapidly absorbs oxygen, but that when the sewage system is sterilized by various methods, generally no oxygen is absorbed. Sterile

sewage is oxidized readily by non-sterile activated sludge and by bacterial suspensions, and less readily by protozoal suspensions. The oxidation is inhibited by cyanide and by methane, but is effected by formaldehyde-treated bacteria still possessing active enzymes although unable to grow upon the ordinary nutrient media. Experiments with identical suspensions of *Ps. fluorescens* irradiated for varying periods with ultra-violet light, and then allowed to oxidize sterile sewage, showed that there is no direct correlation between the rate of oxidation and the number of viable bacteria. It is concluded that although living bacteria oxidize sterile sewage readily, dead bacterial cells, which still retain their enzymic activity, are also active in this respect. Both activated sludges and the contact filters are likely to contain a large number of dead cells in addition to those that are living, a fact which illustrates the importance of the above findings in the processes of sewage disposal works.

Dr. H. Damm (Kiel) : *Die bakteriologischen Vorgänge bei der biologischen Reinigung von Molkereiabwasser.*

Die biologische Reinigung des Molkereiabwassers auf aerobem Wege lässt sich ohne Geruchs- und Fliegenbelästigung nach 2 Verfahren durchführen :

(1) Mittels des zusätzlich belüfteten geschlossenen Tropfkörpers,

(2) Mit Hilfe des Belebtschlammverfahrens.

Das Belebtschlammverfahren ist noch bei höheren Abwasserkonzentrationen durchführbar als das Tropfkörperverfahren. An einer 40 Ltr. fassenden Laboratoriumsanlage wurden eingehende Studien über die Abbauvorgänge beim Belebtschlammverfahren durchgeführt. Wie beim städtischen Abwasser findet innerhalb der ersten 20 Min. eine Adsorption von Abwasserkolloiden statt, die je nach der biologischen Zusammensetzung des Schlammes verschieden gross sein kann. Nimmt man den Gesamtstickstoff als Kennzahl, so kann die Adsorption sogar mehr als 50 prozent betragen. *Die Adsorption ist zurückzuführen auf die Overflächenwirkung* der Zooglöa-bildenden Bakterien und somit biologisch bedingt. Der Abbau selbst wird bewirkt durch ein ineinandergreifendes System Lactose- bezw. Lactat-abbauender Bakterien und Pilze einerseits und proteolytischer Bakterien andererseits. Bei geeigneter Wahl der Versuchsbedingungen kann man erreichen, dass die ersten Abbauprodukte der Milcheiweisskörper sofort wieder zum Aufbau junger Organismen verwendet werden. In diesem Falle tritt *kein Nitrit oder Nitrat im gereinigten Abwasser auf.* Unter diesen Verhältnissen stellt die Abwasserreinigung also eine Überführung nicht lebender organischer Substanz in lebendige Organismen da. Diesen Schluss kann man auch aus der Schlammvermehrung ziehen, die während einer 8-stdg. Reinigungsperiode stattfindet. Die Schlammvermehrung kann bei einem Abwasser mit 1 prozent Milchbestandteilen bis zu 20 Vol. prozent betragen.

Durch besondere Züchtung gelang die Bildung eines Schlammes, der aussergewöhnlich wenig Protozoen und Fadenbakterien enthielt und doch eine gute Reinigungswirkung hatte. Es ist somit der Schluss berechtigt, dass diese beiden Organismengruppen zur Abwasserreinigung nicht unbedingt notwendig sind. Zu demselben Ergebnis gelangte auch *Liebmann* (Leipzig) beim Studium der Bedeutung der Protozoen bei der Selbstreinigung stehenden Abwassers. Die Fadenbakterien scheinen aber neben den Zooglöabildnern eine nützliche Rolle bei der Bildung schnell ausflockenden Schlammes zu spielen. Schlecht absetzender Schlamm (Blähschlamm) zeigt ein übermässig starkes Auftreten von Fadenbakterien. Der Milchzucker ist hierbei ohne Bedeutung. Im Experiment kann man bei Molkereiabwasser Blähschlamm häufig durch Anwendung zu grosser Schlammmengen (mehr als 20 prozent) erzeugen. Als auslösender Faktor tritt dann häufig die Belüftung hinzu. So treten mitunter die Fadenbakterien auf, wenn neben der erwähnten grossen Schlammmenge di ezugeführte Luftmenge gerade noch so gross ist, dass eine aerobe Reinigung möglich ist.

Dr. R. W. Butcher (Alresford).

Conditions of growth in rivers may give a clue to some of the causes of bulking. First, since *Sphærotilus* can grow in the presence of carbohydrate with the addition of nitrate as well as in the presence of proteins alone, cases can obviously arise where a high content of carbohydrate at a certain stage might cause bulking, though bulking can also arise under other circumstances.

There appears to be only one stage at which *Sphærotilus* grows, because often this organism has not reached its maximum growth until an effluent has been decomposing for some time.

One of the compounds which inhibits the growth of *Sphærotilus* is common salt, and though a high concentration is required to prevent growth altogether, smaller amounts might inhibit it sufficiently to stop bulking.

Miss Annie Dixon (Rothamsted).

Indicator protozoa do show the purity of the sludge, and on a percolating filter the types of protozoa show the stage of purification. Gross feeding ciliates occur on the top layers and pure feeding forms at the base.

Dr. E. H. Richards (Rothamsted).

Prof. Smit's view that sugar is not responsible for the bulking of sludge is confirmed by experiments at Rothamsted on the purification of beet sugar factory effluent. No bulking was observed when solutions containing 0.1 per cent. sucrose were oxidized by a sludge

originally derived from domestic sewage. On the other hand, the sludge obtained by treating 1 per cent. milk at ordinary temperatures has frequently bulked very badly, but similar sludge aerated at 30° C. has not shown any tendency to bulk over a period of 5 months. It is not suggested that heat is a practical cure for the disease but it seems probable that some factor which inhibits bulking is developed at a temperature above normal.

Prof. Jan Smit (Amsterdam), objected to the view expressed by Mr. Barritt that the microscopic appearance of the sludge served as an index of its effectiveness, because that appearance was controlled solely by the strength of the sewage.

With reference to Dr. Wooldridge's communication regarding the effect of KCN on the oxygen uptake, the speaker asked if it was possible for the disinfectant to make a selection from the bacteria, so that not only their numbers were reduced, but their character was changed.

Mr. N. W. Barritt (Rothamsted).

Judging from the papers of Prof. Smit and Dr. Damm, it would appear that the occurrence of *Sphærotilus* in activated sludge is more frequent on the continent of Europe than in England. Its development can easily be accounted for by a high strength of sewage liquor, but a cure is more difficult to prescribe. Once a group of organisms becomes dominant, it tends to persist long after the causative factors cease to operate, so that the use of a more dilute sewage might take a longer time to effect its removal than starting afresh to build up a new sludge. This feature of sludge organisms works both ways, and it is hoped to make use of the persistence of dominant organisms by means of a system of restoration or specialized re-conditioning.

It would appear to be always sound practice to work with weak liquors whenever possible, so as to favour the development of organisms towards the bottom of the list given in my paper.

In reply to Dr. Parker, the experiments of Williams are not concerned with rate of sedimentation but with the coalescence of molecules into discrete particles capable of being removed by centrifugation or filtration. I do not think any purely physical process is known to terminate entirely on cooling from a temperature of 20° C. to 10° C. such as was the case in Williams' experiments.

Dr. Wooldridge seems to attach much importance to the activities of the non-viable bacteria. This appears to me to be hardly consistent with the present discussions taking place in Section 7, on the enzyme adjustments which living cells make towards their substrates. Presumably dead cells would not be able to make these adjustments, which would be necessary during the changes in composition occurring in sewage.

Dr. W. R. Wooldridge (London).

In answer to Dr. Parker, bacteria-free protozoa were obtained from Dr. Muriel Robertson and were subsequently kept on a medium consisting of hay infusion, glucose, glycerine and various salts. Throughout the investigation they were tested for the presence of bacteria by frequent cultivation on suitable nutrient media. Certain bacterial dehydrogenases can be isolated from the cell, but so far their use has seemed inadvisable. Owing to the necessity for an oxygen-hydrogen carrier to link the oxidase and dehydrogenase systems, the use of whole cells is more convenient.

Prof. Smit's suggestion that cyanide, acting in a mixed sewage system, may allow only the actively oxidizing organisms to grow, is not supported by experiment with pure suspensions of such organisms. Their oxygen uptake is inhibited by the cyanide as readily as by sewage. Protozoa are not inactive, but are probably less active than bacteria as judged by the numbers found in samples. Some oxidizing power has been demonstrated with suspensions of protozoa in sewage.

In reply to Dr. Barritt, the part played by dead bacterial cells is emphasized, not because they are more active than live cells, but because of the greater bulk of dead cells to be found in sewage, and because of the value of dead cells in the experimental demonstration of enzymic activity.

DISCUSSION *on* RECENT ADVANCES IN FERMENTATION INDUSTRIES

Chairman : Prof. R. E. Buchanan (Ames, U.S.A.).

Prof. T. Chrzaszcz and **Dr. J. Janicki** (Poznań), **Joint Openers.**

Since 1933 a pronounced change of our views upon the mechanisms of alcoholic fermentation and glycolysis has taken place. The results of these investigations have an influence not only upon the alcoholic fermentation industries, but necessitate also a revision of our hypothesis concerning other processes of fermentation.

Alcoholic Fermentation.—In distilleries, attention should be paid to the suggestion of using either potatoes or grain without cooking these products under pressure. Instead, a mechanical disintegration and a subsequent cooking under normal or lower than atmospheric pressure should be used. Other suggestions pertain to the use of topinambur, chicorz and carobbean for alcohol manufacture.

In order to transform starch into sugar the suggestion has been made to use certain bacteria as well as malt or moulds. The nutritive value of the nitrogenous constituents of wort is said to be increased by the addition of proteolytic enzymes of animal origin. Attempts were made to render the fermentation process

more vigorous by the addition of active carbon or by the withdrawal of the resulting ethyl alcohol by means of *iso*amyl alcohol introduced at the bottom of the fermentation vats. The *iso*amyl alcohol, while rising through the liquid, extracts the ethyl alcohol from the fermenting medium and is allowed to flow from the top of the vessel.

Furthermore, the last few years have witnessed many new experiments in the construction of distilling apparatus in order to obtain refined alcohol of a high concentration, or even absolute alcohol. In the rectified distillate one can still find amino or organic sulphur compounds, which lessen the taste value of the product. The quality of the final product depends upon several factors, the most important of which are the quality of the raw materials, the degree of care in working, and the content of alcohol in the distilled product.

Yeast production.—Sugar beet juice and also a solution of wood sugar have been employed in addition to molasses for the production of yeasts. The nutritive value of the medium, usually strengthened by added mineral salts, was further improved by the addition of soya flour or some special preparation made from certain nitrogen materials by cooking with acids under high pressure.

The suggestion was made that multiplication of yeasts might be advantageously carried out in wort of different concentrations. For example, yeasts were at the beginning kept only in a plain mineral salt solution, to which molasses was gradully added. Also worts of changing sugar concentrations were used, whilst the addition of fermented wort toward the end of the multiplication process was suggested, with the object of providing alcohol as yeast food. For the aeration of the wort, air with a CO_2 content of 0·5–3 per cent. was suggested, whereby the carbon dioxide was drawn from vats with fermenting worts.

Some special growth materials are needed for the growing of yeasts and these materials are secreted in the roots of sprouting barley. Much interest has been caused by the production of yeast possessing an increased fermentative power or a higher keeping quality, especially at higher temperatures. In order to attain increased fermentative power addition of different chemicals has been made to the finished yeasts, whereas a better keeping quality results from the addition of substances which produce firm jellies with the interstitial water of the yeast. These are dried out later at a low temperature. Experiments were also conducted with the object of obtaining from beer yeast, by means of a special additional fermentation process, yeasts possessing good baking requirements.

Malt and beer.—In the production of malt numerous suggestions were made regarding the storage of barley and malt in appropriate silos; also additional methods of combating insects were suggested. Interesting discussions have been carried on to find methods, which would permit the quality of barley and of malt to be judged in

advance, as well as the quantity and behaviour of amylase in the grain as compared with the finished malt. Many investigations have also been carried out in respect of hops, permitting us now to estimate better their quality and especially their antiseptic power.

The problems of filtering a hot beer wort, the rapid separation of hops and the aeration of the beer wort with clean air in closed vats are still subjects of investigation. And, furthermore, problems such as that of the keeping quality of beer, methods of pasteurization, as well as the methods of cleaning beer bottles, continue to remain important problems in this branch of industry.

Wine.—In wine production we find numerous suggestions for separating a part of the water found in wine or must, in order to obtain a product of a higher alcohol content. On the other hand some workers are endeavouring to obtain stronger wines by fermenting the must with adequately strong yeasts. The brilliancy of wine is attained by filtering the product through special filters, or the addition of suitable chemical agents. With regard to the production of sparkling wines, we find the suggestion of atomizing the wine into closed spaces charged with carbon dioxide. And there still remains the possibility of obtaining a wine, possessing the characteristics of an old wine, by adding suitable chemicals to the young product.

Production of acetic acid by fermentation.—Few important changes have occurred in this branch of the fermentation industry. The changes are concerned mainly with the conditions under which oxygen is introduced to the acetifiers. Methods for improving the flavour of vinegar by the use of special worts have also been made subject of inquiry.

The clarification of the vinegar can be effected either by filtering through a filter or by the addition of a suitable mixture of chemicals.

Lactic acid production by fermentation.—Many suggestions have been advanced with regard to the preparation of lactic acid. The simplest way is to use a solution of properly prepared molasses. Drained sugar-beet choppings, or straw previously softened by cooking with alkalies may also be used, as well as other raw materials containing polysaccharides, followed by the addition of suitable bacteria.

The formation of lactic acid, which plays an important rôle in the fermentation of cucumbers, sauerkraut and in the preparation of silage as feed for livestock, has to be controlled and regulated during the process of fermentation originated by appropriate species of bacteria.

Production of propionic acid by fermentation.—A certain number of patents give particulars of the production of propionic acid. Different carbohydrate materials have been suggested for the preparation of the wort. The fermentation should be conducted by special bacteria at a temperature of 30° C. The yields amount

to 45–70 per cent. of propionic acid, 30–40 per cent. of acetic acid, and 1–10 per cent. of butyric acid.

The production of glycerine by fermentation.—In the glycerine fermentation industry the main attention is paid to the quality of the apparatus for the distillation of glycerine.

Butyl alcohol and acetone produced by fermentation.—As raw materials, molasses or sugar-beet worts are being mainly used. These worts are fermented by different strains of *Clostridium saccharoacetobutylicum*, or similar bacteria, in the presence of neutralizing agents. A potato mash may also be used, since this contains starch and a pentose (xylose), which, after sterilization, ferment easily with suitable bacteria. The products of fermentation are composed mainly of *ca.* 75 per cent. butyl alcohol and *ca.* 25 per cent. ethyl alcohol.

Butyric acid fermentation.—The production of butyric acid was investigated from several points of view, and it was found that various carbohydrate-containing materials can be used, provided tbat the reaction is kept slightly alkaline and that the acids developing during fermentation are neutralized progressively. The necessary nitrogen compounds can be obtained from yeasts which for a few hours initiate a preliminary alcoholic fermentation of the prepared wort. Then butrytic acid bacteria are introduced into the wort and the fermentation goes on at a temperature of 40–60° C., whereby autolysis of the yeast takes place and the necessary nitrogenous materials for the bacteria are thus formed. Different strains of bacteria are in use for this fermentation, sometimes with the addition of lactic acid bacteria, which hydrolise the nitrogen compounds. The results of fermentation are mainly butyric acid. and acetic acid, and sometimes a small amount of propionic acid The yield of acids amounts to 55 per cent.

Citric acid fermentation.—The best results are obtained by conducting the fermentation with a 20 per cent. solution of saccharose with a small addition of mineral salts and a very slight addition of iron. The main problem consists in choosing a suitable mould. The strongest ones are found among the group of *Aspergillus*. Better results are obtained by inoculating with spores than by working with ready-grown mycelia. The sugar solution should be changed every 7–11 days. The fermentation temperature is *ca.* 30° C. The mould degenerates after a certain length of time and its action becomes weaker. In order to restore its former activity, the mould has to be put through a process of reactivation for which purpose different methods are being used.

Gluconic acid production by fermentation.—Worts containing *ca.* 25 per cent. glucose, maltose or mannose with added mineral salts and also slight amounts of nitrogenous compounds are saturated with oxygen and subjected to a fermentation by acetic acid bacteria at a temperature of *ca.* 28° C. The yield of gluconic acid is *ca.* 100 per cent.; gluconic acid is also formed by different

moulds at a temperature of 35°–30° C. The yield from sugar is *ca.* 90 per cent.

Kojic acid by fermentation.—Kojic acid is formed by suitable moulds through fermentation of different sugars in solutions containing no nitrogen. Using 20 per cent. sugar solutions kept under a pressure higher than atmospheric we obtain a yield of up to 30 per cent. of kojic acid. In mannite solutions kojic acid is also formed by different bacteria of the acetic acid group.

***Prof. P. Lindner** (Berlin) : *Der mutmasslich älteste Gärungserreger,* Pseudomonas Linderni *Kluyver ; seine Entdeckung und praktische Verwendbarkeit.*

Wie Kluyver hervorhebt, konkurriert dieser Gärungserreger mit den kräftigsten Hefen und kann bis 10 prozent Alkohol in einer konzentrierteren Zuckerlösung bilden. Trotzdem benützt man ihn zur Herstellung eines alkoholfreien Bieres, das als " Soma " und " Neosoma " von der Brauerei Schwechat bei Wien in den Handel gebracht ist. Das Neosoma enthält 500 Millionen Keime pro ccm. und dient medizinischen Zwecken. Das Soma ist durch Filtration von dem Bakterium befreit. Das Getränk wird aus 13 grädiger gewönlicher Sudhauswürze mit etwas modifiziertem Maischverfahren hergestellt und enthält pro Liter 116 g. Extrakt mit 62 g Maltose, 47 g Dextrin und 0.47 Vol. prozent Alkohol. Die Glukose und Saccharose ist durch das Bakterium vergoren und ein Teil des gebildeten Alkohols assimiliert. Die Erzeugung erfolgt in einem ungekühlten Raum neben dem Sudhaus und ist in 2-3 Tagen vollendet. Die Erzeugungskosten sind selbst bei kleiner Anlage um ca 40 prozent billiger, als die der Normalbiererzeugung. Volkswirtschaftlich von Bedeutung ist, dass 80 prozent des Extraktes der Würze als Maltose und somit als Nährsubstrat erhalten bleibt im Gegensatz zum Normalbier, bei welchem diese Anteile in Alkohol und Kohlensäure übergeführt werden. Mit der Herstellung dieses Getränkes ist das Lindnersche Bakterium in seine alte Rolle, die es vor Urzeiten bei den primitiven Gärungen in den Tropen gespielt hat, zurückgeführt, nur ist die Sicherheit des Erfolges garantiert und das Anwendungsgebiet auf alle Länder ausgedehnt.

Mit der industriellen Erzeugung des Soma ist aber auch die Bakterie selbst greifbar geworden für andere Anwendungsgebiete. Als solche sind die Milchwirtschaft, die Bäckerei, die Tabakgärung und die Einsäurung von Grünfutter in Angriff genommen, ferner die Vergärung von Agavensäften, Mosten, Zuckerrohrsäften.

Der Begriff Hefe ist in dem altindischen und babylonischen Schrifttum noch nicht bekannt. Man sorgte für das sofortige Verzehren der frisch gärenden Masse. Die Geschichte der Gärung muss einmal revidiert werden auf Grund der Entdeckung des bakteriellen Gärungserregers. Bei der Annahme, dass nur Hefe Gärung erzeugt, bleiben uns viele Ueberlieferungen einfach unver-

ständlich, so die gute Bekömmlichkeit der frisch gärenden Getränke. Nachdem Kluyver gezeigt, dass die Gärungsbakterie, die als Tm, (Abkürzung von *Termobacterium mobile*) allgemein bezeichnet wird, keine Fuselalkohole erzeugt, ist das Rätsel gelöst, und wir werden vielleicht einmal zu jenen alten Gewohnheiten wieder zurückkehren unter wissenschaftlichen und technischen Sicherungen.

Prof. R. Lieske (Dessau, Germany): *Umsetzung brennbarer Gase durch Bakterien.*

Es ist bereits seit längerer Zeit bekannt, dass verschiedene Bakterien in der Lage sind, brennbare Gase z.B. Wasserstoff, Kohlenoxyd und Methan mit Hilfe von Sauerstoff zu oxydieren, biologische Vorgänge, die in der Natur zweifellos von Bedeutung sind für die Beseitigung brennbarer Gase, die bei den verschiedensten Zersetzungsprozessen auftreten. Neuere Untersuchungen ergaben nun, dass brennbare Gase auch bei völligem Ausschluss von freiem Sauerstoff durch Bakterien umgesetzt werden können. Es wurden Bakterien gefunden, die Kohlenoxyd mit Wasserstoff zu Methan und Wasser reduzieren. Es wurde ferner festgestellt, dass Bakterien Kohlenmonoxyd mit Wasser in Kohlensäureanhydrid und Wasserstoff überführen können, und dass das auf diesem Wege entstandene Gemisch von Kohlensäure und Wasserstoff weiter in Methan und Wasser umgewandelt werden kann. Es ist daher möglich, sowohl aus Kohlenoxyd wie aus Kohlensäure auf biologischem Wege Methan zu gewinnen. Genauere Untersuchungen ergaben, dass als Zwischenprodukt bei der biologischen Umsetzung von Kohlensäure mit Wasserstoff zu Methan Essigsäure entsteht. Die beschriebenen Prozesse können praktisch dazu verwendet werden, Leuchtgas, das je nach der Art der Herstellung verschieden grosse Mengen von Wasserstoff und giftigem Kohlenoxyd enthält, auf biologischem Wege dadurch zu entgiften, dass man das Kohlenoxyd mit Hilfe von Bakterien in Methan umwandelt, was ohne wesentliche Verminderung der Heizkraft des Gases möglich ist. — Die interessante Frage, ob die Bakterien, die sich in fast allen Steinkohlenflözen finden, mit der Entstehung von Methan in den Bergwerken (schlagende Wetter) in Zusammenhang stehen, konnte noch nicht endgültig geklärt werden.

Prof. A. M. Buswell (Urbana, Illinois): *Fermentations yielding Methane and Hydrogen.*

A study of the anaerobic fermentation of 91 pure substances, including carbohydrates, alcohols, acids, aldehydes and ketones in both the aliphatic and aromatic series, in the writer's laboratory and numerous earlier and contemporary reports of other workers are summarized. It has been shown that the fermentation is an anaerobic oxidation-reduction reaction involving water and in which there is bacterial catalysis. Carbon dioxide, methane and

hydrogen appear to be primary products, the hydrogen combining with a portion of the carbon dioxide to give additional methane and water. Organic acids, resulting from alpha oxidation, are indicated to be intermediates in the reaction. Thermo-dynamic calculations show that if water is the only source of oxygen besides that in the compound undergoing fermentation, carbon dioxide and methane are the end-products that might be expected since the greatest decrease in free energy occurs with their formation. The general equation :—

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \longrightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)\ H_4$$

has been found to fit the experimental data closely.

The progressive changes in composition of gases evolved during fermentation has been used as a means for determining the course of the reaction. This method is advantageous, since relatively small weights of material can be determined with considerable accuracy and it is not necessary to withdraw a sample of the fermentation mixture for analysis, or at most a very small sample will suffice. Application of the method to the fermentation of dextrose, lævulose, sucrose, and starch indicates that aliphatic acids resembling butyric are first formed in the fermentation and later undergo decomposition to carbon dioxide and methane. The amounts of hydrogen produced from different carbohydrates indicate that there is some difference between the manner of fermentation of dextrose and lævulose, as compared with sucrose and starch.

Prof. Teizo Takahashi (Tokyo) [Communicated by Prof. Teijiro Yabuta (Tokyo)] : *Recent Advance in Fermentation Industries, with Special Reference to Gluconic and Citric Acids Production by Fermentation Process.*

The industry of organic acid production by fermentation is a rather recent one, although many fermentations were used before the dawn of civilization.

Boutroux[1] isolated from culture solution of *Mycoderma aceti* on glucose an acid which two years later he recognized as gluconic acid. Brown[2] confirmed bacteriologically the formation of this acid by *Bact. aceti* and *Bact. xylinum.* Other organisms having this action are *Bact. kützingianum, Bact. pasteurianum, Bact. rancens, Bact. acetigenum, Bact. vini acetati, Bact industrium,* etc.

Molliard (1922) separated the acid as the calcium salt from cultures of *Asp. niger* on sucrose, and since then the acid has been recognized as a rather common product of mould fermentation. Sakaguchi showed the production of gluconic acid by *Asp. wentii,* which gave 50 per cent. of the theoretical yield. May and Herrick[3]

[1] *Compt. rend. acad. Sc.,* 1878, **86**, 605.
[2] *J. Chem. Soc.,* 1886, **49**, 172 and 432.
[3] *J. Biol. Chem.,* 1928, **77**, 185.

investigated the better yield of acid by ***Penicillium luteum purpurogenum***, which gave up to 64.7 per cent. of the theoretical yield and may have a practical application in industry. Hermann[4] isolated from Japanese Kombucha special bacteria named *Bact. gluconicum*, which produce an abundant quantity of gluconic acid. The acid formed from glucose reached 80 per cent. According to May and Herrick, however, the fermentation induced by this organism is quite slow and apparently has little industrial significance. On the other hand, *Bact. koshigaki* and allied varieties isolated by us from fruits seem to have industrial possibilities. Thus, *Bact. koshigaki var. rosea* in 6 days produces 93.5 per cent. of the theoretical yield of gluconic acid from yeast water+glucose.

The discovery of the microbiological production of citric acid was made by Wehmer,[5] who recognized the formation of the acid from sucrose by a mould, to which he assigned the name *Citromyces*. Zahorski[6] (1913) patented the manufacture of citric acid by black *Aspergilli*, and Currie[7] investigated the factors controlling the fermentation by a selected strain of *Aspergillus niger*. Although many moulds have since been reported as citric acid formers, only *Citromyces* and the black *Aspergilli* have been successfully used for the industrial production of the acid.

K. Sakaguchi[8] in our Institute found that certain strains of *Aspergillus oryzæ*, which are employed widely in the fermentation industries of this country (e.g. in the manufacture of Sake or rice wine, alcohol, Soy sauce, Tamari sauce, and Miso or fermented Soy beans) produce considerable quantities of citric acid, and he has succeeded in preparing this acid on an industrial basis.

Dr. H. E. Goresline (Geneva, U.S.A.) : *Recent Wine Investigations*

Research work has been undertaken by the U.S. Department of Agriculture on the microbiology, biochemistry and technology of wine-making with a view to improving the quality of American wines, making their production more economical, and developing new outlets for surplus agricultural products. Studies have been made on the pasteurization of musts and wines, and under conditions in New York a lower temperature than ordinarily used was found to prove effective. Yeasts have been collected from most of the major wine-producing districts of the world and have been used in wine production studies, both for dry still wines and sparkling wines. It has been demonstrated that most of the high purity sugars offered on the American market are suitable for the secondary fermentation of sparkling wines and for the dosage.

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[4] *Bioch. Zeits. Bld.*, 1928, **192**, 1.

[5] *Beitr. z. Kennt. einh. Pilze.* H. 1. Hannover, 1893.

[6] U.S. Patent No. 1,066,358.

[7] *J. Biol. Chem.*, 1917, **31**, 15, 203.

[8] *J. Agric. Chem. Soc.* Japan, 1928, **4**, 129: *ibid.*, 1931, **7**, 748, and *ibid.*, 1932, **8**, 265, 433 and 531.

Research is being done on the use of Florida and Texas citrus fruits in the production of citrus wines and brandies. These products have distinctive qualities and provide an outlet for surplus fruit products. Wines and brandies have been produced in California from passion fruit and certain citrus fruits. The passion fruit products have outstanding qualities as to aroma and flavour.

Wine research projects are being carried out by other agencies in the United States, especially in California and Michigan. The general trend in the wine industry is toward more modern machinery, methods and scientific control of winery operations.

Mr. S. Dattilo-Rubbo (London), asked Prof. Buswell what sources of waste carbohydrate were used in his studies of gas productions and which proved to be the best. Would it not be possible to effect a preliminary hydrolysis of carbohydrate with dilute 1 per cent. sulphuric acid? The resulting solution, containing the hydrolysed sugars and suitably neutralized, could be run into the plant and allowed to ferment.

Dr. S. H. Edgar (Norwich), enquired about the nature of the apparatus used in the American fermentation industry.

Prof. A. M. Buswell (Urbana), answered questions on details of the operation of continuous methane fermentation by referring those interested to the appropriate literature, as facilities and time were not available for him to answer these queries.

Prof. Jan Smit (Amsterdam), asked Prof. Lieske whether calcium carbonate changed the line of fermentation, because it took out the acetic acid.

Prof. Buswell was asked about the maintenance of the plants he described and about the quality of the end products.

Prof. R. Lieske (Dessau).

Sind die aus Kohle isolierten Bakterien dieselben, welche Kohlenoxyd reduzieren? Nein, es handelt sich um verschiedene Formen.

Die Zugabe von Calcium-Carbonaten ist bei der Kohlenoxydproduktion zur Neutralization der freien Säure vorteilhaft, der Reaktionsverlauf wird dadurch nicht verändert.

Prof. R. H. Hopkins (Birmingham).

It has often been stated that the specific flavour, or at least a contributory element in the flavour of a wine, results from the use of the corresponding species or race of yeast. Do such yeasts lose this power after use for a few fermentations in a different must or juice? How far is flavour a function of yeast "type" and how far one of environment, chemical composition of juice, etc.?

Has any relationship been observed in the case of wines or fruit juices between the "cooked" pasteurization flavour and the presence of air, dissolved or otherwise, in the bottle at the time of pasteurization?

SUBSECTION 3—SOIL MICROBIOLOGY.

DISCUSSION *on* THE PHYSIOLOGY OF NITROGEN-FIXING ORGANISMS AND THE BIOCHEMISTRY OF NITROGEN FIXATION

Chairman: Dr. H. G. Thornton (Rothamsted).

Dr. C. Stapp (Berlin), **Opener**: *Zur Physiologie stickstoffbindender Mikroorganismen.*

In der mikrobiologisch-bodenkundlichen Forschung ist den stickstoffbindenden Organismen unzweifelhaft eine hervorragende Bedeutung zuzuerkennen. Hinsichtlich ihrer Physiologie interessiert einmal die Abhängigkeit der Fixationsgrösse von Ernährungs- und anderen Faktoren, zum andern der Fixationsvorgang selbst.

Auf Grund der Untersuchungen von Bortels, Birch-Hirschfeld, Schröder, Konishi und Tsuge, Burk und Mitarbeitern, Rippel u.a. dürfte die Humuswirkung fruchtbarer Böden auf *Azotobakter*-Entwicklung und Stickstoffbindung in den wesentlichsten Zügen als ursächlich geklärt anzusehen sein. Danach beruht diese sehr wahrscheinlich auf der Anwesenheit geringster Mengen bestimmter Mineralien wie Mo oder V und von Kolloiden, die organischer und auch mineralischer Art (kolloidal gelöste Metalloxyde) sein können.

Nach Bortels (unveröffentlicht) sind die wirksamen Konzentrationen von Mo verschieden und auch für die einzelnen *Azotobakter*-Arten unterschiedlich. Für *Azot. chroococcum* z.B. liegt der Wirkungsbereich des Mo (berechnet auf das Element nicht auf das Salz) etwa zwischen 0,0000001 prozent und 0,3 prozent, der des V etwa zwischen 0,00000003 prozent und 0,005 prozent. Für *Azot. vinelandii* ist der Konzentrationsbereich des Mo der gleiche, dagegen ist die Maximaldosis des V bereits bei etwa 0,0001 prozent erreicht.

Bei der Prüfung des Einflusses von Mo, V und W auf *Azotobakter* bei Gegenwart von gebundenem Stickstoff (Natriumnitrat, Ammonsulfat, Asparagin, ca 0,02 prozent) in Luft in einer 2 prozentigen Glukosenährlösung, der jeweils 0,001 prozent Na_2MoO_4, 0,00002 prozent $NaVO_3$ oder 0,0004 prozent Na_2WO_4 zugesetzt waren, ergab sich bei einer Versuchsdauer von 6 Tagen und einer Bebrütungstemperatur von 29° C. folgendes:

Ohne die Spurenelemente hatte in keinem Falle eine Fixation atmosphärischen Stickstoffs stattgefunden, im Gegenteil bei Nitrat- und Ammonsalzzusatz sogar ein deutlicher Stickstoffverlust (—2, 8 bis —3, 9 mg N auf 100 ccm. Nährlösung).

Mo wirkte bei Nitrat- und Ammonsalzzugaben ziemlich gleichmässig: Beschleunigung der Nitrat- bzw. Ammonstickstoffassimilation und Bindung atmosphärischen Stickstoffs (+6, 6 bis +7, 2 mg N). Bei Asparagin als zusätzliche Stickstoffquelle bewirkte, Mo keine wesentliche Ausnutzung des organischen Stickstoffs, dagegen fand eine sehr starke Stickstoff-Fixation statt (+28, 2mg. N).

V zeigte eine ganz andre Wirkung. Die Aufnahme des Nitratstickstoffs war nur geringfügig, die Stickstoff-Fixation aber deutlich (+8, 9 bis+9, 0 mg N). Die Aufnahme von Ammonstickstoff war nicht gefördert, es hatte auch keine Stickstoffbindung stattgefunden. Bezüglich der Verwertung des Asparagin-Stickstoffs verhielt sich *Azotobakter* in Gegenwart von V ähnlich wie bei Gegenwart von Mo, die Menge fixierten Stickstoffs erreichte aber nicht die Höhe wie bei diesem (+17, 8 bis +18, 4 mg N).

W überraschte in seiner Wirkung noch mehr. Es förderte wie Mo die Nitratreduktion sehr stark, dabei trat Stickstoffverlust ein (−3, 7 bis −4, 3 mg N). Auf die Ammonstickstoffassimilation wirkte W beschleunigend, mit gleicher aber schwächerer Verlusttendenz. Auf die Aufnahme des Asparagin-Stickstoffs war W ebenso ohne Einfluss wie auf die Aufnahme des atmosphärischen Stickstoffs.

Die Wirkung unteroptimaler Molybdän- und unteroptimaler wie optimaler Vanadiumkonzentrationen bei der Stickstoffbindung wurde durch W erhöht.

Durch Ca wurde unter optimalen Bedingungen in Luft nicht nur die Aufnahme des Nitratstickstoffs, sondern auch die des atmosphärischen Stickstoffs beschleunigt, eine Feststellung, die mit den Schlussfolgerungen Burks und seiner Mitarbeiter über die bevorzugte Rolle des Ca bei der Stickstoffbindung nicht ohne weiteres in Einklang zu bringen ist.

Im gleichen Sinne wie *Azot. chroococcum* wurde auch *Bac. asterosporus* durch Mo und *Bac. amylobacter* durch Mo und V gefördert.

Nach Bortels und Rippel (unveröffentlicht) fördert Mo und ebenso anscheinend V auch die Stickstoffbindung der Knöllchenbakterien innerhalb ihrer Wirtspflanzen (ausserhalb derselben ist auch durch die Gegenwart dieser Spurenelemente keine Stickstoffbindung zu erreichen !).

Es ist nach Bortels (unveröffentlicht) sogar möglich, den *Azotobakter*-Gehalt Mo-armer Böden durch Mo-Düngung zu erhöhen.

Damit wäre also gezeigt, dass dem Mo (vielleicht auch dem V) in der Physiologie der gesamten Gruppe der stickstoffbindenden Bakterien eine besondere Bedeutung zukommt.

Die Entwicklung und im Zusammenhang damit die Stickstoffbindung von Azotobakter ist aber wie durch mehrjährige Untersuchungen in Dahlem gezeigt werden konnte auch in stärkstem Masse abhängig von der jeweiligen Wetterlage. Während eines Hochdruckes vermag *Azotobakter* ausgezeichnet zu gedeihen, bei

Tiefdruck kann es dagegen zu einer vollständigen Entwicklungshemmung kommen. Es ist möglich aus der Wetterkarte ungefähr herauszulesen, wie die *Azotobakter*-Entwicklung an einem bestimmten Ort zu einer bestimmten Zeit beeinflusst ist. Welche Einzelfaktoren hierbei jedoch ausschlaggebend sind, ist zur Zeit noch unbekannt. Es braucht z.B. nicht der augenblickliche örtliche Luftdruck zu sein, auch magnetische oder luftelektrische Einflüsse kommen nicht in Frage, die Luft als solche oder die sog. Höhenstrahlen lassen ebenfalls keine Beziehungen zur *Azotobakter*entwicklung erkennen. (Dieser Witterungseinfluss ist aber in Zukunft bei allen Versuchen mit *Azotobakter* unbedingt zu berücksichtigen.)

Der Chemismus der Stickstoffbindung ist trotz der darüber vorliegenden umfangreichen Literatur noch keineswegs restlos geklärt. Die meisten Forscher, voran S. Winogradsky, vertreten die Ansicht, dass Ammoniak das erste Zwischenprodukt bei der Stickstoffbindung sei. Das hielten ursprünglich Meyerhof und Burk auch für wahrscheinlich. Burk und Mitarbeiter kamen aber dann zu der Überzeugung, dass das Primärprodukt zwar Ammoniak oder Harnstoff nicht unähnlich, jedoch nicht mit einer der beiden Verbindungen identisch sein werde ; sie konnten in Übereinstimmung mit Roberg zeigen, dass Ammoniak erst unter oxydativer Desaminierung eignen Zellmaterials entsteht. Obwohl sie auch in neuesten Untersuchungen Ammoniak als erstes Intermediärprodukt nicht nachzuweisen vermochten, geben sie doch die Möglichkeit der Entstehung wieder zu. Von Blom wird das Hydroxylamin, von Endres ein Oxim als erstes Zwischenprodukt angesehen. Virtanen und Mitarbeiter, die bei Untersuchungen an Erbsen die Ausscheidung von Asparagin- und Diamidocapronsäure aus den Knöllchen nachgewiesen haben, vermuten, dass die Asparaginsäure aus Oxalsäure über das Oxim entsteht. Ein abscbliessendes Urteil darüber, welche Argumentation hier die richtige ist, lässt sich zur Zeit nicht geben, die Bildung des Ammoniaks als Primärprodukt bei der Stickstoff-Fixation hat jedoch die grösste Wahrscheinlichkeit für sich, bedarf aber unbedingt noch der experimentellen Bestätigung.

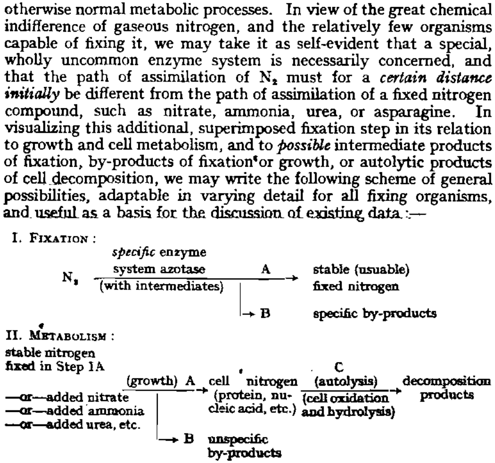
Es besteht kein Zweifel, dass die Stickstoff-Fixation auf enzymatischem Wege erfolgt. Burk und Mitarbeiter führen den Nachweis eines solchen besonderen Enzymsystems bei *Azotobakter* indirekt. Nach ihnen verbindet sich die Nitrogenase, das spezifische Enzym innerhalb des *Azotobakter*systems mit charakteristischer Affinität direkt mit Stickstoff, wobei als Hilfsstoffe das Ca, das auch durch Sr ersetzbar ist, und Hydroxylion angesehen werden. Mo resp. V sollen erst in der zweiten Phase des Bindungsprozesses in Aktion treten. Da das Ca, wie oben erwähnt, aber nicht nur die Stickstoffbindung sondern auch die Aufnahme gebundenen Stickstoffs fördert, scheint die dem Ca von Burk zugesprochene besondere Stellung bei der Stickstoff-Fixation zumindest etwas erschüttert. Bach, Ermol'eva und Stepanian, die den direkten Nachweis des Enzyms in *Azotobakter*zellen erbracht haben wollen, geben an,

Stickstoffgewinne mit dem Press-Saft erhalten zu haben, die so hoch sind (z.B. 276 g Gesamtstickstoff pro g Glukose), dass Zweifel an der Richtigkeit der Analysenwerte berechtigt erscheinen. Es sind also auch bezüglich der enzymatischen Vorgänge weitere Untersuchungen notwendig.

Dass andere freilebende Bakterien als die der *Azotobakter-*, *Bac. amylobacter-* und *Bac. asterosporus*-Gruppe angehörenden ebenfalls die Fähigkeit besitzen, nennenswerte Mengen Stickstoff zu binden, ist bisher unbestätigt, das Gleiche gilt für Aktinomyceten und Pilze, wobei von letzteren vielleicht die Mykorrhizapilze auszuschliessen wären.

Prof. Dean Burk (Washington, D.C.) [Communicated by Prof. P. W. Wilson (Madison, Wisconsin)]: *Criteria of Chemical Mechanism in Nitrogen Fixation by Living Forms.*

The chemical mechanism of nitrogen fixation by various symbiotic and non-symbiotic organisms may be regarded as involving a specific series of chemical reactions whereby free gaseous N_2 is converted into some form or forms of utilizable fixed nitrogen. The series of chemical reactions is carried out over and above otherwise normal metabolic processes. In view of the great chemical indifference of gaseous nitrogen, and the relatively few organisms capable of fixing it, we may take it as self-evident that a special, wholly uncommon enzyme system is necessarily concerned, and that the path of assimilation of N_2 must for a *certain distance initially* be different from the path of assimilation of a fixed nitrogen compound, such as nitrate, ammonia, urea, or asparagine. In visualizing this additional, superimposed fixation step in its relation to growth and cell metabolism, and to *possible* intermediate products of fixation, by-products of fixation or growth, or autolytic products of cell decomposition, we may write the following scheme of general possibilities, adaptable in varying detail for all fixing organisms, and useful as a basis for the discussion of existing data:—



The organic matter and salts necessary in Steps I and II are not specified, nor are possible reversible reactions. The by-products of steps IA and IIB, and the decomposition products of Step IIC, if and when occurring, would be of greatest interest extracellularly. As a phyoenzyme[1], the action of azotase in Step I is normally synchronized with Step II, but freedom of the enzyme from growth limitation, and extracellular isolation, are eventual aims if indeed not already accomplishments.[2]

The problem of the chemical mechanism of fixation is that of determining the nature of Fixation Step I, its enzymes, its intermediates, and its products. Until fixation Step I can be separated from Metabolism Step II, in a manner receiving general and unqualified recognition, it will be necessary to employ the most rigid standards of logic in avoiding the many pitfalls consequent upon the fact that the normal physiology and metabolism of an organism confuses every finding bearing upon its ability to fix nitrogen. It is essential, in attempting to prove any proposed chemical path or mechanism, to observe all or as many as applicable of the following, usually neglected criteria. (1) Comparative studies should always be made between cultures growing, and likewise previously grown, in free and fixed nitrogen. (2) Where commonly desirable, various forms of fixed nitrogen should be studied, such as nitrate (oxidized), ammonia (reduced), and asparagine (organic). (3) Differences obtained with free and fixed nitrogen should be interpreted with due allowance for unspecific effects caused by differences in rates or amounts of growth. (4) N_2 should at times be temporarily eliminated, often with replacement by an inert gas, to demonstrate its true necessity in relation to some other process claimed as specific. (5) Any proposed intermediate should ordinarily be capable of serving as a nitrogenous nutrient. (6) Specific excretion products should preferably be obtained in amounts approximating or greatly exceeding the quantity of cell nitrogen. (7) Specific inhibitors claimed should be subject to (1), (2), (3) and (4), just as any specific intermediate.

Analysis of the published experiments of Kostyschev,[3] Winogradsky,[4] Endres,[5] Blom,[6] and others show that the conclusions drawn from much excellent previous experimental work have been commonly vitiated through failure to apply some or all of these criteria to the data.

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[1] *Ergeb. Enzymforsch.*, 1934, 3, Chap. 2.

[2] *C. r. Acad. Sci. U.S.S.R.*, 1934, I, 1, 22.

[3] *Zeit. physiol. Chem.*, 1926, 154, 1; 1931, 198, 105.

[4] *Ann. Inst. Past.*, 1932, 48, 269; *C. r. Acad. Sc.*, 1933, 197, 209; *Soi Science*, 1935, 40, 59.

[5] *Naturwiss.*, 1934, 22, 662; *Ann. Chem.*, 1935, 518, 109.

[6] *Zbl. Bakt.*, II. 1931, 84, 60.

Prof. P. W. Wilson and **Prof. E. B. Fred** (Madison, Wisconsin) : *Studies in the Mechanism of Symbiotic Nitrogen Fixation.*

Application of physical-chemical techniques to the study of nitrogen fixation by red clover in association with *Rh. trifolii* provides a method for investigations of the mechanism of this biological process. Since 1932, research has been directed toward solution of the pO_2 and pN_2 functions. The major conclusions reached through this work are summarized in this report.

(1) The pO_2 *function.*—Growth and fixation of free nitrogen by the symbiotic system appear to be essentially independent of the partial pressure of oxygen between 0.05 and 0.40 atm. ; in atmospheres with a pO_2 below 0.05 or above 0.40 atm., both growth and fixation decrease. Plants given combined nitrogen have a pO_2 function similar to that of plants fixing nitrogen. Because of this similarity it is probable that molecular oxygen is not directly involved in the fixation reaction but influences the latter only indirectly through the oxidative mechanism of the plant plus bacteria system, e.g., influence on the respiration of carbohydrates.

(2) *The* pN_2 *function.*—The pN_2 function of clover plants fixing nitrogen is similar to the activity-substrate concentration curves of many other enzyme reactions, resembling in general a Langmuir adsorption isotherm. At low pN_2 (below 0.10 atm.) the fixation increases with pN_2 but soon reaches a maximum above which the fixation is independent of the pN_2 of the atmosphere. On the other hand the pN_2 function of plants using combined nitrogen is a straight line parallel to the abscissa, that is, the assimilation of combined nitrogen is independent of the pN_2 of the atmosphere throughout the range studied. The Michaelis or dissociation constant of the enzyme-substrate complex appears to be in the neighbourhood of 0.05 atm.

The response of inoculated plants fixing atmospheric nitrogen to the pN_2, detailed in the foregoing, holds true whenever the nitrogen is : (*a*) not replaced, i.e., plants grown under reduced pressures ; (*b*) partially replaced with oxygen, within the limits indicated in (1) ; (*c*) entirely replaced with helium or argon. However, if hydrogen is used to replace the nitrogen, the fixation is no longer independent of the pN_2 above 0.10 atm. but decreases with decreasing pN_2 throughout the range studied (0.04 to 0.80 atm.) This inhibitory effect of hydrogen (or of an impurity in the hydrogen used) appears to be restricted to plants using elemental nitrogen, that is, the effect is specific for the fixation reaction. Much work has been directed toward establishing this latter point in conformance with the criteria suggested by Burk in the foregoing paper. The evidence bearing on this question may be summarized as follows :

(*a*) Regression coefficients of lines formed by plotting final nitrogen content against pN_2 in the atmosphere have been shown to be significantly different from zero in

case of inoculated plants fixing nitrogen but not in the case of plants using combined nitrogen.

(*b*) Analysis of variance of experiments in which replicate treatments were used show that the difference in means (final nitrogen content) of plants grown in atmospheres of various pN_2 (or pH_2) is significant only in the case of inoculated plants using elemental nitrogen.

(*c*) The effect of hydrogen can be established for plants fixing nitrogen if rate of fixation is used rather than final nitrogen content.

(*d*) Inoculated plants showing inhibition of growth in the presence of hydrogen recover if hydrogen is removed or if combined nitrogen is supplied in the presence of hydrogen.

The inhibiting effect of hydrogen may arise from the gas itself or from an impurity. The gas used in the majority of the experiments was commercial electrolytic hydrogen (99.5 per cent.), the chief impurity being oxygen. This has been further purified by use of chemical chains including H_2SO_4, NaOH, $KMnO_4$, $AgNO_3$, CuCl, lead acetate, and activated charcoal without loss of the inhibiting factor. Further experiments are in progress concerned with these questions of specificity of effect for fixation reaction and possible impurities in the gas; upon completion of these, a detailed report of the work will be published.

Dr. H. G. Thornton (Rothamsted, England): *The Stimulation of the Legume Host Cell Growth by Nodule Bacteria.*

In legumé nodules, healthy symbiosis resulting in nitrogen fixation seems to be intimately related to the continued growth of the distal meristem cap of the nodule. When this ceases, parasitism by the bacteria tends to set in.

Thus, when lucerne nodules, bearing incipient nodules, are supplied with 0.05 per cent. of sodium nitrate, the meristem cap soon ceases to grow and its cell-walls become remarkably thickened. This change is followed by evidences of starvation in the central infected tissue, and in most cases the bacteria actively destroy the central nodule cells.[1]

Lack of a persistent distal meristem may in part explain the failure of " non-beneficial " strains of nodule bacteria to benefit the host plant. Nodules produced on red clover by such a strain showed early disappearance of this meristem, often followed by destruction of the nodule tissue by the bacteria.

Despite the apparent importance of healthy meristem tissue, the causes of its formation and maintenance are as yet unknown. Cell division occurs in tissue not yet invaded by the bacteria, whose secretions probably contain a diffusible growth stimulant.

[1] Thornton, H. G. and Rudorf, J. E., *Proc. Roy. Soc. Ser. B.*, 1936, 120, 240

TABLE.

EFFECT OF THE SECRETIONS OF NODULE BACTERIA UPON THE DEFORMATION AND MEAN LENGTH OF LUCERNE ROOT-HAIRS.
(The Standard errors are based on replicate cultures.)

Treatment	Root-hair deformation			Mean Root-hair length in μ (20 Root-hairs per culture)	Number of replicate cultures
	Number of Root-hairs examined	Per cent. deformed	Number of replicate cultures		
Control ; no secretions	2,616	$6 \cdot 16 \pm 0 \cdot 79$	8	$140 \cdot 8 \pm 13 \cdot 3$	4
Fresh secretions of nodule bacteria	901	$30 \cdot 04 \pm 7 \cdot 11$	4	$304 \cdot 4 \pm 40 \cdot 4$	4
Bacterial secretions heated to 120° C.	893	$49 \cdot 0 \pm 6 \cdot 97$	4	$295 \cdot 8 \pm 11 \cdot 5$	4
Reprecipitated gum	894	$15 \cdot 8 \pm 2 \cdot 93$	4	$208 \cdot 7 \pm 37 \cdot 9$	4
Gum-free secretions	747	$42 \cdot 5 \pm 8 \cdot 44$	4	$218 \cdot 1 \pm 47 \cdot 1$	4
Gum-free secretions after acid treatment	806	$27 \cdot 3 \pm 8 \cdot 2$	4	$231 \cdot 7 \pm 39 \cdot 7$	4

The secretions of nodule bacteria also stimulate the root hairs of the host plant to characteristic deformed growth which, in lucerne, is accompanied by increases both in the number and mean length of the root hairs. The substance here active is contained in the sterile secretions obtained by filtering a suspension of nodule bacteria, and it may be separated from the bacterial gum by precipitating the latter with acetone or alcohol. Activity is not destroyed by heat, but is greatly reduced by hydrolysis. Further investigation of the properties of the active substance is needed before it can be identified with or related to other growth promoting substances, such as the auxins or "pantothenic acid," which latter growth stimulant is claimed by McBurney, Bollen and Williams[2] to be produced by nodule bacteria. The action of the secretions seems to depend on the equilibrium of available carbohydrate to nitrogen in the root hairs, since the supply of nitrates to the roots tends to inhibit root hair deformation, while this inhibition can be overcome by the simultaneous supply of dextrose. It remains to be determined whether the bacterial secretion that stimulates the root hairs of legumes also stimulates cell-division in the nodules and maintains the activity of the distal cap.

Prof. A. I. Virtanen (Helsinki).

Recent research in this laboratory has led to results which, for the first time, enable us to draw definite conclusions regarding the

[2] *Proc. Nat. Acad. Sci.*, 1935, **21**, 301.

mechanism of symbiotic nitrogen fixation. Some years ago we showed that a powerful excretion of nitrogenous compounds takes place in inoculated, but otherwise sterile, quartz sand cultures of leguminous plants. The rate of this excretion was found to be highest at an early stage of growth, before flowering, showing that the excretion was not a result of a protein breakdown in the nodules. The excreted N-compounds have since been extracted from the sand with cold water and were found to contain chiefly *amino nitrogen.* The content of NH_2-N in the extract has been as high as 98–99 per cent. of the total N. The excreted N-compounds are thus chiefly amino-acids.

Further work on the excreted amino-acids showed that they consisted of *aspartic acid* and *lysine.* Since the protein compounds in the nodules and roots contain numerous different amino-acids, it became increasingly clear that the excreted amino-acids were not products of a protein breakdown. No amino-acids were excreted into the sand from uninoculated legumes, grown on nitrate nitrogen. This showed that the excretion is a function of the nodules. The excreted amino-acids are to be regarded as the primary amino-acids formed in the symbiotic nitrogen fixation.

A closer analysis of the water extract from inoculated pea cultures revealed the presence of nitrite nitrogen, particularly after the evaporation of the extract. The nitrite content of the quartz sand cultures, before the extraction, was either nil or very low, so that the nitrites must have been formed during the extraction and particularly during the evaporation. Further work showed that the formation of nitrites was ascribable to *oximes* which were excreted from the nodules into the sand. The amount of oximes has usually been about 1–2 per cent. of the total N ; in some cases, however, the percentage of nitrites has amounted to 10 per cent. of the total N. Oximes have never been found in sterile, uninoculated legume cultures, growing on nitrate or ammonia nitrogen.

The above results are best explained by assuming that the first compound to be formed in the symbiotic nitrogen fixation is *hydroxylamine* which then reacts with *α-keto-acid,* forming oximes. In view of the fact that about one-half of the total N in the excreted material is aspartic acid-N, it is justifiable to assume that this acid is formed over oxalacetic acid, thus :

$$
\begin{array}{l}
CO_2H \\
| \\
CO + NH_2OH \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
\longrightarrow
\begin{array}{l}
CO_2H \\
| \\
C{=}NOH + 2H_2 \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
\longrightarrow
\begin{array}{l}
CO_2H \\
| \\
CH \cdot NH_2 \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
$$

The formation of oxalacetic acid from sugar over succinic, fumaric and malic acid is known to take place in living cells, so that the

synthesis of aspartic acid in the leguminous root nodules can be traced back to sugar and atmospheric nitrogen. The mechanism of the biological nitrogen fixation can thus for the first time be followed up to the production of an amino-acid.

Another explanation for the synthesis of aspartic acid, which seemed very plausible during the early stage of the research, was its formation from fumaric acid and ammonia. This assumption, however, became less probable as it was found that neither the nodule bacteria nor the nodules themselves contain the enzyme aspartase. Moreover, the formation of oximes supports the hydroxylamine hypothesis.

Dr. A. C. Thaysen (Teddington) asked if there was any information to show that the principles of nitrogen fixation differed between *Bacterium radicicola* and *Azotobacter.*

Mr. R. A. Altson (Kuala Lumpur, F.M.S.).

Observations made with parallel cultures of a strain of *Azotobacter* from a Malayan soil and a strain of *B. radicicola* isolated from a species of *Calapogonium* may have some bearing on the question of the relationship of the nitrogen-fixing mechanisms in non-symbiotic and symbiotic organisms. On nitrogen-free mannitol agar the growth of the *Azotobacter* was abundant, whereas that of the nodule organism was much reduced. On mannitol agar, containing nitrogen in the form of potassium nitrate or asparagine, the reverse effect was obtained. This would seem to suggest the existence of some difference in the physiology of the two groups in relation both to nitrogen fixation and to the utilization of combined nitrogen.

Dr. H. G. Thornton (Rothamsted) asked what was the minimum concentration of H_2 that checked N-fixation. The proved inhibitory action of rather large amounts of H_2 and of simple N compounds did not prove that such products, if present at any one time in minute amounts, might not play some part. The speaker suggested that Virtanen's results might be affected by the activity of bacteria outside the plant. Legume roots secreted a substance, possibly Allison and Burk's co-enzyme R, which stimulated considerable growth of the nodule bacteria outside the plant.

The effects of molybdenum and vanadium upon nitrogen fixation was a remarkable advance in our knowledge. It was interesting to hear that Prof. Stapp had found soils so deficient in these elements that their further addition produced an improvement in legume growth. This was paralleled by the case of certain soils now found to be deficient in boron to an extent damaging to plant growth.

Prof. A. I. Virtanen (Helsinki).

Gratuliere Herrn Prof. Stapp zu den schönen Arbeiten über die Wirkung des Mo und V auf die N-Bindung der Azotobakterien.

Betreffend die Wirkung von Agar möchte ich bemerken, dass Agar schon in sehr kleinen Mengen sehr stark das Wachstum von *Aspergillus* fördert (Virtanen u. Pulkki, s. Suomen Kemistilehti-Acta Chemica Fennica, No. 2–3, 1933). Die Wirkung von Agar ist also ganz unspezifisch und erstreckt sich vielleicht auf alle aeroben Mikroorganismen.

Wilson and Fred's quantitative determinations of the influence of pN_2 and pO_2 on the functions of the legume bacteria are very interesting. However, our investigations concerning the effect of oxygen on the growth of legumes and the function of the nodule bacteria do not support the view expressed in the abstract of Wilson and Fred's work that "plants given combined nitrogen have a pO_2 function similar to those using free nitrogen." In our experiments inoculated peas grew extremely poorly in sterile N-free water cultures when the roots were entirely submerged, while under similar conditions uninoculated peas grew much better on ammonium sulphate. Hence the effect of O_2-concentration is more pronounced when the plants receive their nitrogen from the nodules than when they grow on combined nitrogen.

In reply to Dr. Thornton's inquiry as to how we have been able to decide whether the excreted N-compounds originate from the nodules or the roots, I wish to say that, when uninoculated peas are grown on combined nitrogen (e.g. NO_3-N) in sterile sand cultures, the amino-acid content of the sand is practically nil. This is clearly seen from the following table :

Extracted amino-N in 8 kg. sand (4 peas)

inoculated	uninoculated	
	with Ca $(NO_3)_2$	Control
180	7·7	3·4

Should the N-compounds be excreted from the roots, an excretion would necessarily take place also when the plants grow on combined nitrogen. I should think that this is sufficient proof for my view. Besides, should the excretion be a result of protein breakdown in the roots, it would be difficult to explain why only two amino-acids are excreted into the sand.

Dr. C. Stapp (Berlin).

Die Wirkung des Agars ist nur eine wachstumbeschleunigende. Auf den Prozess der Stickstoffbindung ist der Agar ohne Einfluss. Er vermittelt als Kolloid wahrscheinlich nur eine bessere Versorgung des *Azotobacters* mit Stickstoff und Sauerstoff *in* der Nährlösung.

Prof. R. E. Buchanan (Ames, U.S.A.) asked by Prof. Wilson why clover was chosen as the legume for intensive study, since clover is usually cross-pollinated and is highly heterozygous, said

that it was possible that other legumes, which are self-fertile and in which homozygous strains are available, might prove more satisfactory. Possibly there might be decrease in plant variability if pure strains are used.

Prof. P. W. Wilson (Madison, Wisconsin).

The interesting effects described by Prof. Virtanen do not appear to be at variance with the observations reported in our paper. We have emphasized that because of the similarities in the pO_2 functions of plants fixing nitrogen and those using combined forms, molecular oxygen *per se* is not directly involved in the fixation reaction. However, this does not eliminate the importance of oxygen acting in an indirect manner ;—studies at our own and at other laboratories have demonstrated the importance of the carbohydrate-nitrogen balance in the plant on the fixation process, and oxygen is undoubtedly concerned in influencing this relationship. In some recent experiments on the effect of pO_2 in the presence of hydrogen we have evidence that the lower break in the curve occurs at a somewhat higher pO_2 in the presence of this gas than in its absence, but this observation holds only for plants fixing free nitrogen. This again suggests existence of an intimate relationship between the oxidative and nitrogen fixation systems. The results reported by Prof. Virtanen possibly arise from the fact that without aeration, insufficient oxygen is available in the nodule to carry on the necessary oxidations accompanying the fixation reaction ; restriction of development would probably not be observed in plants supplied with fixed forms of nitrogen since assimilation of these would not be confined to the root system as appears to be the case with free nitrogen.

In reply to Dr. Thornton's question regarding quantity of hydrogen necessary to establish inhibition, it is difficult to establish inhibition at low concentrations of hydrogen since variability in the plants would mask any small effects. To date, 0·20 atm. (lowering the pN_2 from 0.80 atm. to 0.60 atm.) is the smallest quantity of hydrogen used, and this has given a definite response. However, if sufficient replicates were taken so that a stable mean could be obtained, smaller pH_2 could probably be shown to give the effect. This is suggested by the fact that if the final nitrogen content is plotted against the pH_2 in the atmosphere, a straight line results which fits the experimental points very closely, i.e., no falling off in the unit effect of the hydrogen is noted as the quantity used decreases.

In reply to Prof. Buchanan's question as to use of clover, red clover was used in the experiments described since its habits of growth are most satisfactory for the technique required. The plants must be grown in a closed system under glass and in certain seasons at rather low light intensities. We have found that of the common leguminous plants clover develops most nearly normal

under these conditions. Likewise, the low nitrogen content of the clover seed is advantageous since this ensures the onset of nitrogen fixation early in the development of the plant—very desirable in the relatively short-time experiments used.

In discussion of questions concerned with identity of enzyme systems in symbiotic and *Azotobacter* nitrogen fixation, one of the purposes of these investigations is to determine in so far as is possible points of resemblance or of difference between the nitrogen fixing systems in leguminous plants and in *Azotobacter*. Because of technical difficulties progress in the symbiotic field is necessarily rather slow ; on the other hand the brilliant researches of Burk and collaborators have established a wealth of detail concerned with the fixation process in *Azotobacter ;* hence proof of the identity of the two systems should further the symbiotic problem decidedly. The evidence to date is not too encouraging ; the KN_2 of the two systems differ, although this may not be so important. Also, the inhibitory effects of hydrogen appear to be restricted to the symbiotic system ; if this is established as specific for the fixation reaction, another point of difference will confront us. It should be mentioned that study of the symbiotic system offers certain advantages with respect to quantity of nitrogen fixed, relative ease of following interrelated reactions as carbohydrate and nitrogen metabolism, and applicability of cytological techniques to the study. It is hoped that investigations in the two fields will supplement one another in a symbiotic manner.

DISCUSSION *on* THE ECONOMIC IMPORTANCE OF THE AUTOTROPHIC AND OF CERTAIN ANORGANOPHILIC BACTERIA

Chairman : Dr. A. C. Thaysen (Teddington, England).

Dr. A. C. Thaysen (*Teddington*), **Opener.**

Various definitions of the term autotrophism as applied to micro-organisms have been and are being given in the microbiological literature. The most exacting is undoubtedly that of Winogradsky who has coined another name for the autotrophs, discussing them as the " anorgoxydants." Winogradsky stresses the importance of the claims that the energy requirements of the autotrophs must be satisfied in their entirety by the oxidation of inorganic substances, and that their carbon requirements must be derived solely from carbon dioxide.

If we accept this definition, the well-known type *Nitrosomonas,* for example, should derive the whole of its energy from oxidation-reduction processes in which ammonia is activated to function as a hydrogen donator, while atmospheric oxygen takes the part of hydrogen acceptor. The various organic carbon compounds

which normally can lay claim to represent the universal source of hydrogen donator in exothermic biological reactions would take no part in the metabolism of *Nitrosomonas* and the other autotrophs, and the carbon metabolism of these forms would have become little more than a side reaction concerned solely with the synthesis of the carbon compounds which are essential for the building up of the cell structure, a reaction wherein available carbon dioxide would function as a hydrogen acceptor.

With our present knowledge it requires a considerable amount of courage to maintain that this interpretation of autotrophism is in agreement with established experimental facts. It is true that Lieske refers in one of his papers to an iron bacterium, *Spirophyllum ferrugineum*, which is capable of synthesizing small quantities of organic carbon compounds. But in a subsequent publication Lieske states that the results of his investigations cannot be taken as proof of the contention that organic carbon sources have been entirely replaced by inorganic sources of energy. Lieske very correctly remarks that much additional work will be required before a final verdict can be given as to whether all and every form of organic carbon supply is entirely useless as a potential source of hydrogen donator in the metabolism of the autotrophs. That complex organic substances are unsuitable, has unquestionably been satisfactorily demonstrated, but there is no writer who has proved that every form of simple organic carbon compound is inhibitory to the growth of the autotrophs. On the contrary, Stutzer and Hartleb declare that the autotrophs with which they were working grew well in the presence of certain organic acids.

If existing knowledge of the metabolic processes of the autotrophs is summarized it is impossible to go further than to say that these types grow best in the presence of inorganic substances and of simple organic carbon compounds. Some of the autotrophs would appear to require the presence of carbon dioxide, but the exact function of this substance has not been demonstrated though it is *known that small quantities of carbon compounds can be synthesized* from carbon dioxide.

In other words, it cannot be claimed that only inorganic substances function as hydrogen donators and acceptors in the energy producing reactions activated by the autotrophs. It would appear that such substances *can* function, but the fact that simple organic compounds frequently favour growth indicate very definitely that these compounds also can be activated to serve as hydrogen donators. Considered in this light the autotrophs can be regarded as a specialized group of all the anorganophilic micro-organisms, the types which prefer their nitrogen and phosphorus requirements to be met by inorganic substances while their carbon requirements are best satisfied by simple organic compounds. The most striking difference within the group would be that the true autotrophs possess the power of activating inorganic hydrogen donators in addition to

simple organic carbon compounds, while the rest of the group is limited to the latter source.

It is in this light that the economic significance of the activity of these interesting micro-organisms is discussed, notably the practical importance of the reduction of sulphates to sulphides, phosphates to phosphine, carbon monoxide to methane and silicates to unknown decomposition products. Next, certain views are referred to which might connect the activity of anorganophilic micro-organisms with such natural phenomena as the deposition of calcium carbonate and the eruption of sulphuretted hydrogen in certain seas, with the formation of medicinal mud at Russian Spas and with the formation of naturally occurring hydrocarbons.

*__Prof. D. Ellis__ (Glasgow) : *The Iron Bacteria.*

There are two associations of économic value in this group—one connected with Leptothrix, Gallionella and Spirophyllum, the other with Cladothrix and Crenothrix. The members of the first group may be pleomorphic varieties of the same species. *Cladothrix dichotoma* is the name of the group species.

Nodule formation in iron pipes. The change is chiefly one of chemical oxidation, the biological activity of Iron Bacteria being only contributary.

The blocking of streams with mucilaginous strands is frequently due to Iron Bacteria.

Depositions of ferric oxide in streams, wells, etc. Iron deposits are frequently composed of dead bodies of Leptothrix, and these organisms are occasionally seen in the living condition ; on the other hand, some deposits have no trace of Iron Bacteria. Our knowledge of reproductive cells of Leptothrix, Gallionella and Spirophyllum is dealt with. Autotrophism is an accessory mode of nutrition, the organisms being mainly saprophytes.

Crenothrix bacteria are of great importance owing to their power of rapid multiplication. The author discusses the following problems : Cheltenham waterworks ; depredations of the organism on the Continent ; developmental history ; multiplication in ultra-microscopic form ; conditions of multiplication ; reproductive methods ; failure of artificial cultures.

Cladothrix dichotoma. This name is used for the group species, though some members are not Iron Bacteria. The author deals with developmental history (most, if not all, species are saprophytes) and artificial cultivation.

The following points will also be discussed : the conditions of multiplication of Iron Bacteria (the nature of the gathering grounds for water of reservoirs is the chief factor for multiplication of Crenothrix and Cladothrix), the nature of their autotrophism, their successful artificial cultivation.

Decay of stone buildings. Not due to activity of bacteria.

Sulphur Bacteria. After defining terms, the economic importance of the elimination of hydrogen sulphide will be considered. The centre of interest is chemical, not biological. Sulphur compounds of lower energy level are absorbed and compounds of higher level are liberated. There is also the question of the autotrophy of Sulphur Bacteria; the morphological features of bacteria of this group have not been well studied, and they have a complicated physiology. Complete knowledge, however, is impossible without information concerning pleomorphism of the group.

It would be desirable to deal with the following problems: chemical and biological oxidation of sulphides; the sterility of decaying stone protected from the atmosphere; the bacterial removal of sulphur from shales; the origin of black mud in estuarine rivers and the connection with sewage pollution; *Beggiatoa alba* as indicator of sewage pollution. Coloured Sulphur Bacteria are not in the same class as chlorophyll organisms.

Mr. H. J. Bunker (Teddington, England): *The Economic Importance of the Sulphate-reducing Bacteria.*

The bacterial reduction of sulphates appears to be exclusively the work of vibrio types related to *V. desulphuricans.* These types are very widespread, and besides occurring in the sea and river muds with which they are commonly associated, they are present in most soils and subsoils, and sometimes even in tap water. Although active under anaerobic or microaerobic conditions, the vibrios retain their viability for prolonged periods of unfavourable aerobiosis.

Many of the activities of these bacteria are deleterious, as in the production of hydrogen sulphide in coal-gas holders, in filter beds, and in wood-pulp and paper manufacture. The decay of stonework and concrete may also be due to these types, particularly in sewage tanks. Here the evolution of hydrogen sulphide arises as much from the reduction of sulphate as from protein sulphur, and the gas appears to be subsequently oxidized to sulphuric acid, resulting in turn in the formation of calcium sulphate in the upper parts of the chamber. That the decay has been observed to be particularly rapid in cases where the water is rich in sulphates, strengthens the view that the sulphate-reducing organisms are responsible. The organisms have frequently been found in the brine at the bottom of oil wells, and it has been suggested that they may be responsible for some of the sulphur in crude oil. There is increasing evidence that these vibrios play a part in the corrosion of iron pipelines in clay and chalk soils. The conditions are favourable, and where corrosion has occurred, not only are the organisms present, but they are in association with iron sulphide. The presence of appreciable amounts of sulphide among the decomposition products is inexplicable solely by the electrochemical theory of corrosion. It has been suggested (von Wolzogen Kühr) that the rôle of bacterial

sulphate reduction is the prevention of kathodic polarization, since the oxygen of the sulphate provides hydrogen-acceptor. At the same time, precipitation of the anodic iron as iron sulphide will prevent polarization at the anode.

The formation of sulphide ore deposits has been attributed to the organisms, and it has been suggested that the natural deposits of sodium carbonate in certain parts of the world arise through the activity of the sulphate-reducers. One investigator attributes in part the deposit of chalk in tropical seas to these types.

Prof. S. G. Paine (London): *The Occurrence of Autotrophic Bacteria in the Stones of Buildings and their Relationship to the Decay of Stone.*

There is difficulty in assessing the importance of autotrophic bacteria in the process of stone decay. It is an easy matter to determine the presence in decaying stone of three groups of autotrophic bacteria: (1) nitrifying, (2) sulphur-oxidizing, and (3) sulphate-reducing. These groups correspond to the normal soil types; no flora specific to stone has come to light. There is a possibility that infection of stone may take place from the air, from soil or from street dust. It is at present an open question whether infection induces decay. On the other hand, decay may be a necessary forerunner of infection, since, while it is easy to see that a stone surface injured from some other cause of decay can form a lodgment for bacteria, it is probable that bacterial colonization would be difficult on an uninjured surface. Having obtained a lodgment, the above groups find suitable conditions for growth and their acidic metabolic products must have a decaying influence on any calciferous stone. It has not been possible to determine whether bacteria have ever been the first cause of decay. In many instances the sporadic nature of the decay and the localized spread of decay from an initial focus has suggested bacterial agency and the efflorescence of calcium sulphate has caused one to look for a source of oxidisable sulphur within the stone. The appearance of a brown stain due to oxide of iron which is frequently to be found associated with sulphate efflorescence lead one to search for the presence of iron pyrites as the source of sulphur. Iron pyrite is not often found in sections of limestones—it has however occasionally been seen in sections of both sandstones and limestones and has been found in considerable quantity in a bromoform sediment of crushed stone. In sections of stone which reveal no crystals of pyrite there is evidence in the cubical form of limonite to show that this originated from pre-existing pyrite. With all this, however, there is so far no proof that decay has ever originated from oxidation of pyrite.

The proof of bacterial action in decay is elusive and remains to be discovered. The chief difficulty encountered lies in the ready oxidizability of sulphurous acid. It is not necessary to invoke the

action of bacteria to produce sulphuric acid from the sulphur dioxide of the atmosphere. On the other hand it is difficult to understand, supposing the decaying action to be purely chemical, why decay should commence, should produce what may be regarded as open sores and then with the surface of fresh stone nicely exposed should come to a complete stop. This is what one so frequently finds and one leans towards the view that such cases of decay are biological. The stoppage of the action could be explained by an alteration in the conditions for growth of an organism much more readily than by an alteration in the conditions for oxidation of the atmospheric gases. I am at a deadlock in my experimentation. A hint from any one of you may point the way to overcome my difficulties. I shall therefore welcome a full criticism and discussion.

Dr. R. W. Butcher (Alresford).

A question to be solved is that of the maximum oxygen pressure at which *Vibro desulphurians* works. Since H_2S is very toxic to fish and in river pollution, when the oxygen gets low, this is very important. Iron compounds may be deposited by algal as well as by other organisms, and I have frequently observed, particularly in soft water, deposits around algal growths. Is it possible that CO_2 is removed from soluble iron salts by assimilation, and that hydrate and oxide are deposited ?

Beggiatoa alba grew at the mouths of sewage effluents in salt water in the Tees estuary, but its place is taken by *Sphærotilus* in fresh water.

Dr. F. M. Muller (Amsterdam).

For the corrosion process produced by sulphate reducing bacteria von Wolzogen Kühr has given the following overall equation :

$$4\,Fe + H_2SO_4 + 2H_2O \longrightarrow 3Fe(OH)_2 + FeS.$$

It follows from this equation that the principal corrosion product is $Fe(OH)_2$ and not FeS. Iron hydroxide, being comparatively soluble, will diffuse away from the pipe, provided that no sulphate reduction with organic substances as H-donators is going on in the surrounding soil and furnishing additional H_2S with which the $Fe(OH)_2$ will react to form FeS.

Dr. von Wolzogen Kühr has pointed out that yet another microbiological process may possibly be active in the corrosion of iron in the soil. This is the hydrogenation of CO_2 to CH_4 by nascent hydrogen carried out by methane fermentation bacteria. It is quite possible that these bacteria are able to carry out this process with hydrogen atoms attached to the surface of the iron, and if such a process exists, it will probably be of importance where iron pipes are lying in marshy soils where active methane fermentation is taking place.

Dr. H. G. Thornton (Rothamsted).

Prof. Paine has given us a clear exposition of a very difficult subject. There is a difference of opinion between biologists and chemists as to the cause of stone decay. Both parties agree that the characteristic flaking of the surface of building stones is immediately due to the circumstance that crystals of hydrated calcium sulphate occupy a greater volume than crystals of calcium carbonate containing equivalent amounts of calcium. The chemists claim that town atmospheres contain sufficient sulphur compounds to produce the observed results by purely chemical action. The question is whether the action is initiated or stimulated by bacterial action. Prof. Paine has shown, rather unexpectedly, that building stones contain sufficient mineral nutrients, such as phosphate and potash, to support growth of sulphur-oxidizing bacteria. The local action of decay has a superficial appearance of biological activity, but, on the other hand, it is difficult to suppose that infection of the surface of a building can be so localized. Moreover, the local lesions in the stone could perhaps be explained as due to centres of weakness in the stone, such as minute cracks which would initiate chemical action. It would seem desirable to examine more minutely the surface and sub-surface of stones which are affected, and of entirely undecayed stones, in the hope of finding such differences.

Prof. S. G. Paine (London).

Iron forms on organisms in all cases by a process of adsorption from soluble iron. The view that CO_2 may be removed, leaving hydrate and finally oxide, is probably correct.

Dr. Thornton is quite correct in stating that sulphur oxidizing bacteria and soil organisms generally are found on the surface of perfectly sound stone. I have to thank Dr. Thornton for the suggestion to look for the essential mineral food constituents in sound and unsound stone.

Answering a question as to whether the crust split off from decaying stone is of unchanged stone composition, that may not be so, as sulphites and sulphates are found in small amounts. It must not be assumed that, in all decay, crusts of this kind are split off. Many instances are known where the surface at the decaying spot just crumbles away until deep lesions are left.

SECTION 5

MEDICAL, VETERINARY AND AGRICULTURAL ZOOLOGY AND PARASITOLOGY.

DISCUSSION *on* THE BIOLOGY OF THE MALARIAL PARASITES OF MAN AND ANIMALS

Chairman : Prof. E. Brumpt (Paris).

Lieut.-Col. J. A. Sinton, I.M.S. (Kasauli, India), **Opener :** *Some Problems in the Host-Parasite Relationship in the Malaria Infections of Man and other Animals.*

Accurate identification of the species of parasite responsible for the infection being studied, is one of the first essentials in carrying out any investigations of these problems.

The recent work on the differentiation of the species of avian and simian plasmodia has done much to advance the study of the biology of these parasites. Although four separate species of human plasmodia are now generally recognized, there is much evidence to support the view that two or more distinct species may be included under the term " malignant tertian parasite." An exhaustive study of this parasite is required in different countries. It is possible that in this way some light may be shed on the problem of blackwater fever.

As it is now definitely proved that different species of plasmodia react differently to therapeutic agents, it is also very important that there should be no doubt about the identity of the commoner species of human malaria parasites.

Marked clinical and other differences have been reported between infections with *Plasmodium vivax*, produced by blood inoculation, as compared with those by mosquito bite. Are these differences due to an increased virulence of the parasite when passed through the insect host, or does the sporozoite have some intermediate stage before it attacks the red blood corpuscle ?

It has now been shown that strains, of what is morphologically a single species of *Plasmodium*, vary considerably in their effects on the host, and in their reactions to therapeutic agents. The fact that the tolerance produced to infection is highly specific for the strain of parasite causing the infection and *not* for a heterologous strain, suggests very strongly that not only has each *species* of *Plasmodium* a different " antigenic " character but that in each species there are many *strains* with heterologous antigenic properties. This fact is helping to elucidate many problems in epidemiology which were previously obscure.

The practicability of isolating large masses of parasite substance from experimental infection in monkeys, has made practicable means for research into the character of such " antigenic " substances and

also of the "toxin" whereby the malarial parasite produces its pathogenic manifestations. This parasite substance should also be useful, not only in researches into the serology and diagnosis of malarial infection, but also in attempts to produce an experimental tolerance to the infection.

Experimental infections in man and monkeys have opened up a wide field of research in relation to the mechanism of tolerance, both natural and acquired, in this disease, and also into the factors involved in both clinical and radical cure of infection by either natural or therapeutic means.

Prof. R. D. Manwell (Syracuse, U.S.A.) : *The Problem of Species as it relates to the Malaria Parasites.*

The question of what constitutes a species is often puzzling, and especially in the case of the malaria parasites. This is particularly true of the malaria parasites of birds, of which at least thirteen new species have been described in the last ten years.

Among the characters used to distinguish species in the past have been, besides the morphology in the vertebrate host, effects on the host cell, length of asexual cycle, host-parasite specificity, reaction to anti-malarial drugs, climatic and geographic distribution, pathogenicity, duration of infection, etc. The question of possible hybridization in the mosquito has not been raised, and yet this is probably one of the most important criteria of species.

Ability to cause a new infection, by superimposing a new strain of malaria parasite upon an old one, has often been regarded as a test of difference in species, especially in the case of birds, but recent work by Gingrich and by the author has shown that species morphologically different may nevertheless produce an immunity to infection with the other. That tests of this sort may be useful in establishing relationships, however, is indicated by the fact that morphologically similar species appear usually to produce an immunity which is mutually protective—such as that of *Plasmodium cathemerium* toward *præcox* or vice-versa, and the immunity following infection with *vaughani*, *hexamerium*, *nucleophilum* and *rouxi*—though the immunity is not always reciprocal or equal in degree and there are exceptions.

Host-parasite specificity, though quite rigid in the human malarias, is much less so in the case of the avian malarias, yet it may be of use in delimiting species even there.

Morphological characters must remain of first importance in describing a species, but they are not always enough, since there is much overlapping between distinct species of avian malaria. Other characters are certainly important, and yet they are often quite variable. It is probable that the definition of a species will not be well understood until the question of whether crossing occurs in the invertebrate host is thoroughly investigated.

Lieut.-Col. S. P. James, I.M.S. (London) : *Biological Problems of* Plasmodium vivax *and* P. falciparum *in the Human Host and Insect Vector.*

Knowledge of the life history of *P. vivax* and *P. falciparum* in the human host and insect vector has increased considerably since it became possible to study the subject in cases of induced malaria, but several problems which are important clinically and epidemiologically still remain to be investigated. The contribution is a statement of some of these problems and a brief record of what is known about them.

Sir Rickard Christophers (London) : *Observations on* Plasmodium knowlesi *Infection in* Silenus rhesus *with Description of some Physico-chemical Properties of isolated Parasite Substance.*

A strain of *P. knowlesi*, obtained through the kindness of the Malaria Survey of India, has been maintained in *S. rhesus* in London for some three years. Virulence, as judged by the course of the disease and the mortality, has not altered after three years' passage by blood inoculation (37 passages). Periodicity of 24 hours has been maintained with sporulation period at about 12 noon in all cases. The time of appearance of parasites after subcutaneous inoculation has varied from 2–9 days, with a mean for 67 animals of 5·7 days. The time has been little influenced by the number of parasites inoculated which has varied from massive doses (·5 cc. of blood containing 2 million parasites per c.mm. to blood negative to microscopical examination). In the latter case the mean was a few days longer. Death in untreated animals is invariable and no animal has survived beyond the 7th day from the first appearance of parasites. Counts of parasites in typical cases dying on the 5th day showed a mean value of 1 ; 5 ; 200 ; 2,000 and 20,000 parasites approximately per 100 fields on the successive days. The actual mean for the last day was 17,200 per 100 fields and as there are thus 4 cycles concerned the rate of increase per cycle works out $\sqrt[4]{17,200}$ or 11·5. The mean number of merozoites formed is given by Sinton and Mulligan (1933) as 8–16, usually 10. There would appear therefore to be little or no destruction of parasites in the primary attack. Animals treated sufficiently to survive attacks show recurring attacks at intervals of about 7 days for a period of about 6 weeks. This is followed by a period where parasites are more or less continually present, but attacks infrequent, up to a mean of about 6 months, when spontaneous disappearance of parasites supervenes with apparent cure and immunity against reinfection.

Quinine in repeated doses of 2 gr. (0·13 g.) is effective in saving animals if treated early. Atebrin is remarkably effective and animals almost moribund can frequently be saved. A dose of 0·1 g. or two doses of 0·05 g. was effective in practically all cases ; 0·05 g. was effective in some cases, but sometimes failed. The effective single dose for animals of 3–5 kilos was therefore 0·1 g.

Isolated parasite substance can be prepared by centrifuging the blood of heavily infected animals and can be washed free from the hæmoglobin of the cell in hypotonic salt. Such substance in suspensions shows acid agglutination phenomena in the absence of electrolyte and alkaline (irreversible) agglutination in presence of electrolyte. Lysis does not occur even with strong acid, but is readily brought about by alkali, a 1/100 suspension being completely lyzed in a concentration of 0·0003 *N*NaOH. Both acid and base are absorbed with *p*H phenomena similar to those shown in absorption of acid and base by red cells (formation of acid and base protein complexes). Quinine base is absorbed with similar *p*H changes and brings about agglutination and partial lysis of the material.

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Prof. N. H. Swellengrebel (Amsterdam) stated that the fact that two species were much alike morphologically was no reason why they should hybridize. Cross-breeding experiments in *Anopheles* have clearly shown that species which were hardly distinguishable did not hybridize. In benign tertian malaria Korteweg's " initial fever " occurred only in individuals who never had malaria in their life. If they had malaria before, either vivax or falciparum malaria, they very rarely showed the syndrome of " initial fever " after subsequent infections. Initial fever, consequently, was a sign of immunity which was not strictly specific.

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Prof. E. Reichenow (Hamburg).

Die Feststellung, dass es bei den Malariaparasiten des Menschen Stämme mit verschiedenen biologischen Eigenschaften gibt, kann vielleicht Licht auf die Frage werfen, ob die Malariaparasiten der afrikanischen Anthropoiden, deren morphologische Uebereinstimmung mit den 3 Arten des Menschen ich schon vor etwa 20 Jahren nachweisen konnte, mit den Parasiten des Menschen identisch sind. Möglicherweise gibt es Parasitenstämme, die nur für den Menschen und solche, die für Mensch und Menschenaffen oder nur für Menschenaffen infektiös sind. Es könnten hier ähnliche Verhältnisse vorliegen, wie etwa in den Beziehungen der Trypanosomaarten *T. gambiense* und *T. brucei* zu einander.—Für die Artunterscheidung der Malariaparasiten erscheint auch die Feststellung der Ueberträger wichtig. Von deu meisten Affenmalariaparasiten kennen wir die Ueberträger noch garnicht ; diese brauchen keineswegs bei allen Anophelesarten zu sein. Denken wir dabei an die Vogelmalariaparasiten, wo ich für eine Art, nämlich *Plasmodium circumflexum* feststellen konnte, dass sie sich 100-prozentig in *Theobaldia annulata*, aber nicht in *Culex pipiens* entwickelt.

Prof. C. Schilling (Berlin).

Dass bei Malaria ein Antigen wirksam ist, hat mein Assistent Neumann gezeigt; ob dieses Antigen mit dem hypothetischen Toxin identisch ist, ist nicht erwiesen. Können Affen als Reservoirs der menschlichen Malaria dienen? Das frühzeitige Auftreten der Gametocyten ist wichtig für die Epidemiologie; Behandlung der ersten Anfälle bei Kindern kann sehr wirksam sein. Bei Versuchen mit pathogenen Protozoën ist zu berücksichtigen, dass Passagen ohne Zwischenschaltung der natürlichen Übertragung die biologischen Eigenschaften der Parasiten weitgehend verändern können; Beispiel, Trypanosomen. Hybridenbildung könnte nur eintreten, wenn im gleichen Augenblick Gameten der beiden Plasmodienarten im Ventriculus des Anopheles vorhanden sind.

Dr. W. Kikuth (Elberfeld).

Eine besondere Eigenschaft der Malariaparasiten im Gegensatz zu Trypanosomen und verschiedenen anderen Protozoen ist die Konstanz der biologischen Eigenschaften. Trotz zahlreicher, über mehrere Jahre sich erstreckende, vorgenommenen Tierpassagen, ganz gleich ob es sich um Blutüberimpfungen oder Übertragung durch die Mücke handelt, ist bei der von uns geübten Technik eine Virulenzsteigerung bzw. Virulenzabnahme der Vogelmalaria (*Plasmodium præcox* und *P. cathemerium*) nicht zu beobachten gewesen. Diese Beobachtungen stehen im Einklang mit denen von Christophers bei *P. knowlesi*. Eine gelegentlich von mir beobachtete Virulenzsteigerung der Malariaparasiten war auf eine gleichzeitige Virusinfektion zurückzuführen. Auch bei jüngeren oder geschwächten Tieren kann eine Virulenzsteigerung vorgetäuscht werden, sie ist aber immer auf den Wirt und nicht auf den Parasiten zurückzuführen. Das Gleiche gilt auch in bezug auf eine Arzneifestigkeit. Weder mit Chinin, noch mit Plasmochin gelingt es eine auch nur andeutungsweise in Erscheinung tretende Festigkeit gegenüber diesen Medikamenten zu erzielen.

Der Heilungsprozess bei der Chemotherapie der Infektionskrankheiten vollzieht sich durch direkte Wirkung des Medikamentes auf die Erreger. Sie wird unterstützt durch bereits vorhandene oder entstehende Abwehrkräfte des Organismus. Die experimentellen Arbeiten der letzten Jahre haben diese Auffassung weitgehend gestützt und begründet. Durch Ausschaltung oder Herabsetzung der natürlichen Abwehrkräfte des Organismus kann gezeigt werden, daß auch die Wirkung chemotherapeutischer Mittel nicht oder nur herabgesetzt zur Geltung kommt. Das Verhältnis dieser beiden dominierenden Faktoren zueinander ist bei den verschiedenen Infektionen nicht immer gleich. Es gibt Erreger, bei denen der direkten Wirkung eines Medikamentes eine grössere Bedeutung zugemessen werden muss als den Abwehrkräften und andere, be denen die natürlichen Heilungsvorgänge im Vordergrund stebei und das Medikament nur unterstützend eingreift. Eine allgemeinn

Regel, die für alle Erreger Gültigkeit besitzt, gibt es nicht. Es lässt sich jedoch behaupten, dass bei natürlichen Infektionen im allgemeinen die Abwehrkräfte die grössere Rolle spielen, während bei den experimentell erzeugten, soweit sie dem natürlichen Infektionsmodus nicht entsprechen, die direkte Wirkung des Medikamentes deutlicher in Erscheinung tritt.

Zu diesen beiden dominierenden Faktoren gesellen sich noch eine Reihe anderer, die einerseits von der Wechselbeziehung zwischen Organismus und Parasiten, von der Virulenz der Erreger und der Resistenz des Organismus, andererseits von der Resorption und Exkretion der Substanzen abhängig sind. Einzelne dieser Faktoren sind uns bekannt, wie z.B. die Lokalisation der Erreger bei den Trypanosomeninfektionen. Handelt es sich um eine Infektion des Blutes, wie sie bei den kleinen Laboratoriumstieren vorherrscht, so wird es durch die direkte Wirkung eines trypanoziden Mittels unter Umständen möglich sein, in wenigen Stunden die Erreger abzutöten. Liegt jedoch eine Affektion des Zentralnervensystems vor, was bei der natürlichen Infektion meistens der Fall ist, so wird es ohne die Mithilfe des Organismus unmöglich sein, eine Heilung zu erzielen.

Ähnlich liegen die Verhältnisse bei *Malaria*. Eine durch Blutübertragung erzeugte Malaria bei Paralytikern, die bekanntlich über schlechte Abwehrkräfte verfügen, ist mit Leichtigkeit mit wenigen Chinindosen rezidivfrei zu heilen, während die natürliche Malaria, die durch das Eindringen der Sporozoiten zustande kommt, bei sonst gesunden Personen selbst durch eine optimale Behandlung hochwirksamer Medikamente nicht mit Sicherheit rezidivfrei geheilt werden kann. Auch hier scheint der Unterschied durch die Lokalisation der Erreger bedingt zu sein. Während bei der Blutübertragung der Entwicklungszyklus der Malariaparasiten auf das Blut beschränkt ist, machen die Sporozoiten, die durch den Mückenstich in den Organismus eindringen, höchstwahrscheinlich ausserhalb des Blutes eine uns noch unbekannte Entwicklung durch, ehe sie die roten Blutkörperchen befallen.

In anderen Fällen haben wir es noch mit vollkommen unklaren Verhältnissen zu tun. So lässt sich z.B. die *Vogelmalaria* bei der Blutübertragung—dasselbe gilt auch für die Piroplasmose des Hundes—gleich zu Beginn der Überimpfung, wenn noch keine Abwehrkräfte vorhanden sein können, mit wirksamen Mitteln mit fast absoluter Sicherheit heilen, während dieselben Mittel nahezu unwirksam sind, wenn man mit der Behandlung erst nach Abklingen der akuten Symptome beginnt, wenn also bereits die nachweisbaren Abwehrkräfte die Oberhand gewonnen haben und wir eine " labile Infektion " vor uns haben. Weder kleine noch grosse Dosen des betreffenden Medikamentes—selbst über Wochen gegeben—haben, abgesehen von der Unterdrückung der klinischen Symptome, anscheinend irgendeinen Einfluss auf die Erreger. Hier würde man durch eine Analyse des Wirkungsmechanismus tiefer in das biologische Geschehen eindringen.

Für die chemotherapeutische Forschung als solche, die der Auffindung neuer wirksamer Substanzen dient, hat sich aber die Auffassung einer direkten Wirkung als heuristisch erwiesen.

Prof. M. Ciuca (Bucarest).

Observations faites au cours de la malaria-thérapie avec une souche de *Pl. knowlesi* sur 220 malades des hôpitaux : Socola et Hop. Central de Bucarest, 30 passages directs d'homme à homme ont été réalisés au cours de 14 mois. Cette espèce est virulente pour l'homme : il existe une immunité acquise qu'on peut mettre en évidence par réinoculation. Il existe également un *certain degré* d'immunité croisée contre les espèces humaines de parasites. Chez 3 malades avec immunité acquise après une première inoculation de *Pl. knowlesi* on n'a pas pu mettre en évidence la présence du parasite par examen du sang (6 gouttes épaisses) ou par inoculation de 15 c.c. de sang au *Macacus rhesus*.

Prof. J. Rodhain (Antwerp).

Le pouvoir pathogène de *Plasmodium knowlesi* n'a guère été expérimenté sur des singes inférieurs Africains. Grace à l'amabilité de Sir Rickard Christophers, j'ai pu disposer d'une souche de *Plasm. knowlesi*, que j'ai inoculée à plusieurs espèces de singes Africains.

J'ai trouvé ainsi que *Papio jubilæus*, un cynocéphale commun dans la savane du Katanga au Congo Belge, est extrèmement sensible à *Pl. knowlesi*. Il s'infecte après 3 à 5 jours et fait une malaria mortelle accompagnée d'hémoglobinurie tout comme le fait *Macacus rhesus*. Au contraire, *Cercocebus galeritus agilis*, constitue une espèce relativement résistante à *Pl. knowlesi* vis-à-vis duquel il se comporte sensiblement comme *Macacus cynomolgus*.

Prof. W. A. P. Schüffner (Amsterdam).

Vielleicht darf man aus Differenzen der Plasmodien-Zahl auf die Anwesenheit von Toxinen schliessen. Quartana erreicht ihr Maximum schon bei *ca.* 20,000 per c.mm., die Tertiana bei *ca.* 100,000, obwohl der Anfall bei Quartana eher heftiger ist. Dies würde darauf weisen, dass Quartana schon mit einem Fünftel der Zahl Parasiten, die wir bei Tertiana sehen, die gleiche Wirkung hervorruft. Ganz anders verhält sich der Tropicaparasit, der sich ungehemmt vermehrt, bis schliesslich alle Erythrocyten befallen sein können : die Todesursache. Der Tropicaparasit würde darnach die geringste, der Quartana die grösste Toxinbildung entfalten.

Prof. F. Mesnil (Paris) s'associe à ce que vient de dire M. Brumpt. Il attire aussi l'attention sur l'intérêt des faits signalés concernant *Plasmodium gallinaceum* ; cette infection intense de

l'oie est très curieuse et est de nature à modifier nos idées sur les champs d'action comparés des hématozoaires extraglobulaires (trypanosomes) qui s'attaquent à des espèces animales très éloignées les unes des autres et les hémocytozoaires (*Plasmodium*, *Piroplasma*) qui étaient considérés comme ayant un champ d'action zoologique très restreint. Le cas du *P. gallinaceum* fait une certaine exception.

DISCUSSION *on* CHEMOTHERAPY—MECHANISM OF DRUG ACTION AND DRUG RESISTANCE

Chairman : Prof. E. Brumpt (Paris).

Prof. H. Schlossberger (Berlin), **Opener.**

Während die Serumtherapie mittels Immunstoffen den infizierten Körper von den Krankheitserregern zu befreien sucht, wird bei der Chemotherapie dieser Heileffekt mit Hilfe spezifisch wirkender chemisch definierter Substanzen angestrebt. Im Gegensatz zur sogennannten Reizkörpertherapie, deren Wirkung bei Infektionskrankheiten in einer unspezifischen Steigerung der Abwehrmassnahmen, also einer vermehrten Leistung der Körperzellen besteht, nehmen umgekehrt die beiden Formen der spezifischen Therapie, die Serum-und die Chemotherapie dadurch, dass sie direkt auf die Krankheitserreger einwirken, dem Organismus gewissermassen Arbeit ab. Erst sekundär kann es beim chemotherapeutischen Heilungsvorgang auch zu vermehrten Immunitätserscheinungen kommen.

Der chemotherapeutische Heileffekt ist ähnlich wie die Antikörperwirkung ein komplexer Vorgang, dessen Mechanismus insbesondere durch die Untersuchungen über Arzneifestigkeit und Interferenzphänomen, sowie über die Verteilung der Chemotherapeutica im Organismus und ihre Aufnahme durch die Parasiten eine gewisse Klärung erfahren hat. Wenn auch die Mehrzahl dieser Forschungen an Trypanosomen ausgeführt wurden, die sich hauptsächlich wegen der weitgehenden Differenzierung ihres Zellaufbaus hierfür besonders gut eignen, so hat sich doch gezeigt, dass die hier gewonnenen Ergebnisse in weitgehendem Masse auch für andere Arten von Krankheitserregern zutreffen.

(1) Die erste Phase der chemotherapeutischen Wirkung ist die Verankerung des Heilmittels durch die Parasitenzelle, wobei neben chemischen Affinitäten (" Chemozeptoren " Ehrlichs) auch physikalische Momente eine Rolle spielen. Bei arzneifesten Parasiten ist nach Ansicht der meisten Autoren infolge einer erblich fixierten Permeabilitätsänderung der Erregerzelle die Aufnahme der betreffenden Substanz und jener Chemikalien, die zur gleichen Gruppe gehören, herabgesetzt oder gänzlich aufgehoben. Demgegenüber sind andere Autoren der Meinung, dass die Arzneifestigkeit auf

einer erhöhten Toleranz der Parasiten oder einer erstmals von Ehrlich angenommenen herabgesetzten Reaktivität ihrer Bindungsgruppen beruht; ihrer Meinung nach führt die durch ein Chemotherapeuticum bewirkte Schädigung empfindlicher Trypanosomen sekundär zu einer vermehrten Aufnahme der Substanz durch die Mikrobenzelle, wodurch Permeabilitätsunterschiede zwischen normalen und resistenten Parasiten vorgetäuscht werden. Sicher ist jedenfalls, dass eine Substanz, die von der Mikrobenzelle gespeichert wird, deshalb noch kein Heilmittel ist, da von den Parasiten auch unwirksame Stoffe aufgenommen werden. Die Beobachtung, dass zwar beim *Trypanosoma lewisi*, welches eine *natürliche Resistenz gegen die meisten Arsenverbindungen besitzt*, nicht aber bei künstlich gegen Atoxyl gefestigten Naganatrypanosomen der Blepharoplast durch Akridinfarbstoffe zum Verschwinden gebracht wird, sowie die Festellung, dass die Festigung gegenüber einem bestimmten Mittel zu einer überempfindlichkeit des Parasiten gegenüber anderen Substanzen führen kann, deutet doch darauf hin, dass beim Festigungsvorgang auch Permeabilitätsänderungen eintreten. In diesem Sinne spricht ferner das sogenannte Interferenzphänomen, das darin besteht, dass Trypanosomen nach Vorbehandlung beispielsweise mit Parafuchsin durch Trypaflavin nicht mehr beeinflusst werden und das offenbar darauf beruht, dass das Trypaflavin nur noch in geringer Menge oder überhaupt nicht mehr in die Zelle hineingelangt.

(2) Die toxische Wirkung der verankerten Substanz kann zur raschen Abtötung (Brechweinstein, 3-Amino-4-oxyphenyl-1-arsinoxyd) oder zu einer langsamen Vergiftung der Parasitenzelle (Germanin) führen und besteht in Störungen fermentativer Vorgänge oder der Fortpflanzung. Es hat den Anschein, dass manche Chemotherapeutica überhaupt nur eine Schwächung, aber keine Abtötung der betreffenden Krankheitserreger herbeiführen. In diesem Falle, sowie bei Verwendung kleiner, zur restlosen Vernichtung sämtlicher Erreger nicht ausreichender Heildosen parasitizider Präparate kann die Sterilisierung des Körpers durch:

(3) Die Abwehrmassnahmen des Organismus (Antikörper, Phagozytose) erfolgen. Sind diese ungenügend oder werden sie vollkommen ausgeschaltet, so bleibt hier der Heileffekt aus. Dass Germanin auf pathogene Trypanosomen in der Glossine oder im Blutegel und Rivanol auf Streptokokken in der toten Maus nicht einwirken, ist vielleicht auf ungenügende oder gänzlich fehlende Immunitätsvorgänge zurückzuführen; auch manche Fälle von "*Therapieresistenz*" gewisser *Erkrankungen des Menschen* (Syphilis, Schlafkrankheit, Malaria) können wohl dadurch bedingt sein. Andererseits ist es sehr gut denkbar, dass z.B. die Heilwirkung der Goldpräparate nicht nur bei Spirochätenerkrankungen, sondern auch bei der Tuberkulose dadurch zustandekommt, dass die Erreger durch die Goldverbindung "sensibilisiert" und dann durch die Immunitätsreaktionen des Körpers abgetötet werden.

Prof. C. H. Browning (Glasgow) : *Mechanism of Drug Action and Drug Resistance.*

Trypanosomes remain the most instructive test objects and by the use of "syringe propagated" infections the drugs effective in the naturally acquired diseases have been discovered. Drug action depends essentially on chemical constitution—the ratio of toxicity for parasite to that for host measuring therapeutic efficacy ; but many factors are involved in this ratio. Certain of these can be elucidated by comparing the action of analogues, e.g., in the styrylquinoline series. Thus, against *Trypanosoma brucei* in mice a subcutaneous injection of styryl-90 [2(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride] possesses very slight prophylactic action, which is gone within a week : on the other hand, styryl-245 [2(*p*-acetylaminostyryl)-6-(*p*-aminobenzoylamino)-quinoline methoacetate] forms a depôt and protects against infection for periods up to a year. The amount of the latter drug in circulation at any time is very minute, however. Hence, other things being similar, rate of excretion is of preponderating importance. The behaviour of different species of host to a particular drug may also be decisive and cannot be predicted ; e.g., the tolerance of horses to styryl-90 and the curability of *T. brucei* in them (Parker) is greater than could be expected from tests in rabbits. Accordingly, the general pharmacological properties of chemotherapeutic agents must be studied. Different species of trypanosomes in the same host do not necessarily show parallel behaviour to several classes of drugs ; e.g., styryl-90 acts powerfully on *T. brucei* in mice but is without effect on *T. congolense*, while compounds of bismuth or antimony and certain other substances have a slight but nearly equal action on both. The views of Ehrlich and his school on the mode of action of trypanocidal drugs have been generally confirmed by several elegant methods ; more attention should be paid to exploring different chemical groupings for therapeutic action since this knowledge is fundamental.

The development of resistance, apparently from accidental absorption of minute amounts of trypanocidal drugs, has been met with twice in strains of *T. brucei*. The resistance was manifest both in rabbits and mice. It is noteworthy that in the latter a transient phase of supersensitiveness of the infection to the therapeutic action of the drugs preceded shortly the development of marked resistance.

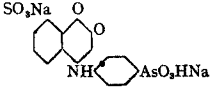
Dr. E. A. H. Friedheim (Geneva) : *Contribution à la chimiothérapie des trypanosomiases.*

L'hypothèse de travail qui a guidé ces expériences a été suggérée par l'observation suivante : Un certain nombre de pigments naturels d'origine microbienne, végétale et animale ont la propriété physico-chimique de systèmes à réduction et à oxydation réversibles. Cette propriété confère à ces pigments la possibilité de jouer le rôle

de catalyseurs des oxydations biologiques, de ferments respiratoires accessoires. Sous l'influence de ces catalyseurs le métabolisme des micro-organismes anaérobies facultatifs, à fermentation aérobie, est profondément modifié : En effet, par la voie de la réaction de Pasteur-Meyerhof la fermentaion aérobie est transformée en respiration. Les pigments naturels, catalyseurs respiratoires, nous permettent donc d'intervenir dans un mécanisme intime de la physiologie microbienne et la question se pose, si cette intervention ne pourrait modifier la fonction pathogène de certains micro-organismes. Des expériences tendant à éprouver cette hypothèse ont montré qu'un pigment bactérien tel que la pyocyanine (une phénazine) et des pigments végétaux tels que le Juglon et le Henné (des naphthoquinones) ont une action faible mais nette sur la trypanosomiase expérimentale de la souris. Disposant ainsi d'un certain nombre de substances naturelles, à constitution chimique connue, à fonction physiologique, pénétrant visiblement dans les cellules, peu toxique pour le macro-organisme et ayant en soi une action favorable, quoique faible, sur la marche de la trypanosomiase expérimentale, nous avons essayé d'employer ces substances comme véhicule d'une action chimiothérapeutique décisive en y introduisant des groupes chimiques d'un effet thérapeutique éprouvé, tel que les acides phényl-arsiniques.

En collaboration avec le professeur F. Ullmann nous avons préparé des arsénicaux du type des flavines, des phénazines et des naphthoquinones.

Des expériences préliminaires ont désigné pour une étude expérimentale approfondie la substance suivante (No. 2654 N)



La dose max. tol. intrapérit. pour la souris est de 0,6 gr/kg.

La dose min. cur. intrapérit. pour la souris est de 0,2 gr/kg.

La dose max. tol. i.v. pour un lapin est de 1,5 gr/kg., pour un homme adulte de 4 gr.

La distribution de cette substance dans l'organisme est dominée par son caractère d'acide sulfonique qui détermine :

(1) une grande solubilité dans l'eau et une insolubilité dans les lipides.

(2) une pénétration rapide dans toutes les humeurs de l'organisme, y compris l'humeur aqueuse et le liquide céphalo-rachidien (substance retrouvée dans le L.C.R. 8 minutes après une injection i.v. de 2 gr. dans un homme de 60 kg.).

Au point de vue clinique ces propriétés se traduisent par :

(1) une inocuité parfaite pour le système nerveux central,

(2) une élimination très rapide par les reins.

Grâce à l'amabilité des autorités britanniques l'action thérapeutique de cette substance a pu être éprouvée au Nigéria sur la maladie du sommeil humaine (*Trypanosoma gambiense*, transmis par *Glossina palpalis*). Le principe du traitement est l'injection intraveineuse quotidienne de 2 à 3 gr. poursuivie pendant 15 jours. L'élimination rapide par les reins excluant tout danger d'accumulation et l'inocuité pour le système nerveux rendent superflue l'observation habituelle d'un intervalle entre les injections. (Il a été trouvé utile de faire précéder le traitement par 3 doses croissantes de 0,5 à 1.5 gr.).

Dans 57 cas ainsi traités les parasites disparurent du sang et des ganglions cervicaux au bout de 1 à 6 injections, en moyenne après 3 injections.

13 cas ainsi traités ont été ré-examinés 2 semaines à 3½ mois après le traitement et furent trouvés exempts de symptômes cliniques et de parasites.

Le sel de calcium de la même substance (2654 C) a montré un certain effet thérapeutique administré par voie buccale.

L'action de 2654 N et C sur la lymphocytose méningée a été favorable dans les 5 cas qui ont permis la ponction lombaire. (Réduction du nombre des éléments cellulaires respectivement de 30 à 4 (21 jours de traitement) ; de 670 à 30 (17 jours de traitement) ; de 132 à 3 (14 jours de traitement) ; de 289 à 176 (15 jours de traitement) ; de 253 à 40 (30 jours de traitement).

Notons que la structure quinoide du produit lui confère les propriétés d'un système d'oxydo-réduction avec un potentiel approximativement au niveau de celui de la pyocyanine. Tout changement apporté à la constitution chimique de la substance ayant une répercussion sur les propriétés d'oxydo-réduction abolit toute action thérapeutique.

Dr. N. von Jancsó (Szeged) : *Chemotherapeutische Wirkung, Oxydationskatalyse und Redoxpotential.*

Vertr. schwebte das Ziel vor, Chemotherapie und moderne Biochemie des Zellstoffwechsels einander näher zu bringen.

Die trypanozide Wirkung dreiwertiger Arsen- und Antimonverbindungen wird im Tierkörper, so wie auch in vitro durch Wasserstoffacceptoren und Oxydationskatalysatoren weitgehend aufgehoben. Es wird eine neue Theorie der Interferenzerscheinungen aufgestellt, die besagt, dass solche Verbindungen Interferenz erzeugen, die thermodynamisch reversibele Redoxsysteme bilden und deren Redoxpotential einem bestimmten Potentialbereich angehört. Die Bestätigung dieser Theorie lieferte die Entdeckung von 17 neuen Fällen von Interferenz, die zeigen, dass die verschiedensten elektroaktiven Körper die Heilwirkung von *m*-Amino-*p*-oxyphenylarsinoxyd oder Fuadin auszulöschen vermögen. Wie die Prüfung von 22 chinoiden Redoxfarbstoffen zeigte, hängt die Wirksamkeit vom Redoxpotential ab : es wirkten nur die 10

Farbstoffe mit einem Potential zwischen E'_0 + *0·115* und — *0·070* Volt bei *p*H 7 : optimal Azur und Toluidinblau. Starke Inter-, ferenzwirkung zeigten natürliche Atmungskatalysatoren, Chinone Phenole, Pyocyanin, Vitamin C, Alloxan, ferner Bluthämin, das in den Flagellatenstoffwechsel atmungsfermentartig eingreift.

Mechanismus der Interferenz : die Redoxsubstanzen wirken auf das Trypanosom und setzen dessen Giftempfindlichkeit herab, indem sie als Wasserstoffacceptoren und Hilfskatalysatoren in die Oxydationsprozesse eingreifen. Dementsprechend erweisen sich diejenigen Farbstoffsystem als interferenzwirksam, die als Atmungskatalysatoren auftreten können und auch bei Vergiftung der Gewebsatmung mit Cyan antagonistisch wirken.

Nachdem bei diesen Versuchen das Atmungssystem der Mikroorganismen sich als wichtiger Angriffspunkt chemotherapeutischer Mittel herausstellte, wurden verschiedene Atmungs- und Gärungsgifte ausprobiert, wobei tatsächlich festgestellt werden konnte, dass *Monobromessigsäure* die Trypanosomen-Blutinfektion abzuheilen vermag. Damit ist es zum ersten male geglückt, eine chemotherapeutische Wirkung biochemisch aufzuklären, da die Monobromessigsäure zweifellos auf die anaerobe Gärungsphase des Zuckerstoffwechsels der Trypanosomen wirkt, indem sie die Bildung der C_3-Körper aus Hexosen verhindert.

Als besonders fruchtbar erwiesen sich biochemische Gesichtspunkte bei der Erforschung des Wirkungsmechanismus der Chemotherapeutika bei Spirochätenkrankheiten. Die Heilwirkung der *Goldverbindungen* bei Recurrensinfektion wird dadurch bedingt, dass das Schwermetall mit den Wirkungsgruppen wichtiger Spirochätenfermente inaktive Komplexe bildet. Betroffen werden hauptsächlich die glykotischen und die am Wachstumsprozess beteiligten Fermente. Die lebenden Spirochäten zeigen die charakteristischen Erscheinungen der reversiblen Fermentinaktivierung, wie sie u.a. bei Proteasen beobachtet werden können. Durch starke Komplexbildner wird das giftige Metall den Fermenten wieder entzogen : durch Glutathion, Cystein oder HCN können die durch Goldverbindungen " getöteten " (unbeweglichen) Spirochäten augenblicklich wieder belebt werden. In vivo heben Sulfhydrylverbindungen die Heilwirkung auf : ein Beispiel für Interferenz bei Spirochäten. Diese Wirkungsart erklärt auch die Beziehungen zwischen Spirochätozidie und periodischem System. Die Resistenz gefestigter Spirochätenstämme wird nicht durch Impermeabilität, sondern wahrscheinlich durch gesteigerte Produktion eines natürlichen komplexbildenden Fermentaktivators bedingt. In Bestätigung dieser Annahme konnten arzneifeste Spirochätenstämme durch starke Sanocrysinbehandlung in vitro vorübergehend in arzneiempfindliche umgewandelt werden, da der Ferment- und Aktivatorvorrat verbraucht bzw. abgesättigt wird.

Dr. W. Kikuth (Elberfeld) : *Wirkungsmechanismus der Medikamente.*

Der chemotherapeutisch eingeleitete Heilungsvorgang vollzieht sich durch eine direkte Wirkung des Medikaments auf die Erreger und geht einher mit einer bereits vorhandenen oder mobilisierenden indirekten Wirkung der Abwehrkräfte des Organismus. Zu diesen beiden dominierenden Faktoren gesellen sich noch eine Reihe anderer, die ineinander greifen, sich gegenseitig unterstützen, bezw. aufheben und die weitgehend von der Wechselbeziehung zwischen Organismus und Parasitismus abhängig sind. Nicht der Streit, ob eine direkte oder indirekte Wirkung vorliegt, sondern die Erkenntnis des komplizierten Zusammenhangs ist wichtig für die Forschung. Die Analyse des Wirkungsmechanismus lässt uns tiefer in das biologische Geschehen eindringen. Für die Auffindung neuer chemotherapeutischer Heilmittel wird man sich jedoch einfacher Vorstellungen bedienen, wobei sich die Arbeitshypothese einer direkten Wirkung als zweckmässig erweist, wie an vielen Beispielen gezeigt werden kann.

Dr. M. Rohonyi (Budapest) : *Catlin, ein neues Chemotherapeutikum zur Behandlung des seuchenhaften Verwerfens und der seuchenhaften Sterilität von Rindern und Schweinen.*

Catlin (cattle=Rind) liegt auf einer neuen chemischen Basis. Es entspricht, den von mir aufgestellten Bedingungen, die an ein wirksames Chemotherapeutikum gestellt werden müssen.

Den Versuchen in der Praxis sind Laboratoriumsversuche *in vitro* und an kleinen Tieren *in vivo* vorangegangen. Der bakterizide Titer des Catlins ist 1 : 1000 (in 1 Minute volkommene Abtötung). Zur Überprüfung des Mittels sind klassiche Tierversuche (von 10 infizierten Tieren 5 behandelten und 5 nicht behandelten) leider nicht durchführbar. Der tierische Körper reagiert auf infektiösen Abort zu verschieden.

Im Laufe der letzten zwei Jahre sind in Ungarn mehr als 3000 Injektionen in natürlich verseuchten Beständen vorgenommen worden. Durch dreimalige subkutane Injektion liess sich das Verwerfen bei infektiösem Abort bedeutend vermindern. Nach zweimaligen subkutanen Injektionen wurde eine 50–75–90—prozentige Trächtigkeit bei durch Bangkeim verursachter Sterilität erreicht. Verabreicht wurden 20 c.cm. bei Rindern in 3-wöchentlichem Abstand.

Schweine erhielten nur 6 c.cm. pro Injektion. Die Dosis muss wiederholt werden, da sonst ein Verwerfen vorkommen kann.

Nach den ermutigenden therapeutischen Erfolgen verspricht Catlin auch ein wertvolles Prophylaktikum bei seuchenhaftem Verwerfen zu werden.

Dr. W. Schulemann (Elberfeld): *Der Mechanismus der chemotherapeutischen Wirkung.*

In seinem einleitenden Referat hat Schlossberger dargelegt, wie wir uns nach dem heutigen Stande der Forschung das Ineinander greifen der direkten Substanzwirkung auf den Parasiten und der körpereigenen Kräfte vorstellen.

Um aber solche pharmako-therapeutische Wirkung ausüben zu können, muss eine Substanz zunächst zu der Zelle oder dem Zellsystem gelangen, auf das sie einwirken soll. Die Resorbierbarkeit der Substanz ist abhängig vom Funktionszustand des Applipkationsortes und von den physikalischen Eigenschaften der Substanz selbst. Diese sind bedingt durch ihre Molekulargrösse und überlagert durch die chemische Konstitution. Sie werden variiert durch die Natur des Lösungsmittels, die Konzentration, Temperatur, Alter der Lösung und Gegenwart anderer Elektrolyte, Kolloide oder indifferenter Stoffe, d.h. — allgemein gesprochen — durch das "Milieu." Darüber hinaus ist für die Qualität und Intensität der Wirkung massgebend, in welcher Form und Menge die Substanz in der Zeiteinheit an den Erfolgsort gelangt. Von wie grosser Bedeutung für die Verteilung einer Substanz körpereigene Stoffe wie z.B. Albumin, Globulin, Fibrinogen usw. sind, haben insbesondere die Arbeiten von v. Jancsó, Bennhold und Sir Rickard Christophers gezeigt.

Sind diese Vorbedingungen erfüllt, so muss die Substanz nun unmittelbar in Beziehung zu der Zelle oder dem Zellsystem selbst treten, auf das sie wirken soll.

Auf welche Ursachen diese mehr oder weniger selektive Verteilung zurückzuführen ist, bleibt eine noch offene Frage. Grenzflächen- bezw. Oberflächenphänomene sind etwa im Sinne der Adsorption, Diffusion, Phagocytose, statischen elektrischen Aufladung usw. zur Deutung herangezogen worden.

Aber es kommt nicht nur auf die Eigenschaften der Substanz bezw. ihrer Aggregate mit Plasmabestandteilen an, denn die Zelle selbst ist keineswegs rein passiv. Wie sie aktiv z.B. durch Änderung ihrer Permeabilität sich dem Eintritt einer Substanz verschliessen kann und damit ihr Wirksamwerden verhindert, haben unabhängig voneinander und mit verschiedenen Methoden Warrington Yorke und v. Jancsó gezeigt.

Eigenschaften der Substanzen und Eigenschaften der Zellen greifen ergänzend und wechselseitig ineinander und überschneiden sich in vielen Fällen.

Über den feineren Mechanismus der Wirkung wissen wir sowohl bezgl. des Einflusses auf körpereigene Zellen, d.h. dem Objekt der Pharmakologie, als auch bezgl. der Einwirkung auf körperfremde Zellen, d.h. dem Objekt der exp.Therapie von Infektionskrankheiten nur sehr wenig. In jedem Falle wird es sich um einen Einfluss auf den Zellstoffwechsel handeln, der eine Funktionsänderung bedingt — im Extrem, um ein völliges Erliegen der Zellfunktion, d.h. um eine Abtötung.

Aber auch hier wieder ist es notwendig, sich vor Augen zu halten, dass Stoffwechsel und Funktion der Zelle wechselseitig ineinandergreifen, sodass Wirkungsdauer und Wirkungsintensität in hohem Masse abhängen von der Stabilität bezw. Labilität der Substanz und den jeweiligen Stoffwechseleigenschaften der betroffenen Zellen.

Abschliessend üben dann noch Weg, Art und Geschwindigkeit der Exkretion bezw. Elimination ihren Einfluss aus, um die Wirkung einer Substanz zu variieren bezw. ganz aufzuheben.

Wir stehen also bezgl. des Mechanismus der Wirkung von und der Resistenz gegen Arzneistoffe einem überaus kompliziert zusammengesetzten Gesamtproblem gegenüber, das nur lösbar erscheint, wenn zu seiner Deutung sinngemäss alle Lehren und Erkenntnisse von Physiologie und Pharmakologie, von Wirt und Parasit herangezogen werden.

Prof. E. Singer (Prague): *Chemotherapie als Spezialfall der Vitalfärbung.*

Chemotherapie wird als Spezialfall der Vitalfärbung aufgefasst und unterschieden zwischen: (1) Mechanismus der Bindung an die Erregerzelle, (2) Mechanismus der chemotherapeutischen Wirkung. Verteilung und Speicherung wird geregelt durch Ladungssinn und Löslichkeitsquotient Fett/Wasser im physiologischen Milieu.

Die Chemotherapeutika sind durchwegs Substanzen von stark negativer Ladung und umladenden Einflüssen des Lösungsmittels leicht zugänglich. Die Bindung erfolgt anscheinend an positive Gruppen der Erregerzelle. Analog lässt sich die Verteilung der wirksamen Elemente im periodischen System erklären. Da bei Naganainfektion Interferenz zwischen Atebrin-Trypaflavin, Phenylphosphinigersäure, Atoxyl und Solganal " Sulfoharnstoff " nicht nachweisbar sind, dürften die aufgenommenen Substanzen schnell an andere Gruppen, (insbesonders SH-Gruppen) weitergegeben, oder die ursprüngliche Verbindung Mittel-Erreger wieder gesprengt werden. Der Bindung von chemotherapeutisch wirksamen Stoffen folgt unmittelbar Störung der fermentativer Tätigkeit und Aenderung in der Ladung der Zelle. Trypanosomen, welche Chemotherapeutika aufgenommen haben, lassen sich leichter abzentrifugieren, was als Herabsetzung der Hydratation aufgefasst wird. Eine Veränderung der Heilmittel durch chemische oder physikalische Einflüsse des Tierkörpers ist unwahrscheinlich. Toxizität und Heilwirkung von Atoxyl, welches durch Lösung in Anti-arsanil- bezw. Anti-benzidin-Serum mit der Arsin- bezw. Amino-Gruppe locker an Globulin gebunden wurde, sowie die Heilwirkung von Atoxylimmunen Mäusen bleibt unverändert. In Trypanosomen aufgenommenes Trypaflavin zeigt Veränderungen in der Stärke der Fluoreszenz und Verbreiterung der Fluoreszenzbande nach Rot. Innerhalb der speichernden Erregerzelle müssen daher Veränderungen des Mittels angenommen werden. Aufnahme eines Chemotherapeutikums und Heilwirkung sind nicht identisch. Sporozoiten

von *Proteosoma præcox* und *Plasmodium vivax* nehmen Akridinfarbstoffe nicht auf, Gameten von *P. falciparum* speichern Atebrin. Der Mechanismus der Heilwirkung, welche durch Aufnahme der Erreger in das Speicherzellensystem eingeleitet wird, bleibt unklar.

Prof. P. Uhlenhuth (Freiburg i. B.) : *Ueber die moderne Antimontherapie bei Tropenkrankheiten.*

Nachdem Uhlenhuth durch die Feststellung der spezifisch spirilliziden Wirkung der organischen *Arsenpräparate* (Atoxyl und atoxylsaures Quecksilber) und den Ausbau des Tierexperiments die Grundlage für die moderne Arsentherapie der Spirochaetenkrankheiten (Hühnerspirillose, Recurrens, Frambösie, usw.) besonders der *Syphilis* geschaffen hatte (1906-1910), die dann in ihrer Weiterentwicklung zum *Salvarsan* (Ehrlich) führte (1910), war er schon seit 1907 bemüht, ähnliche organische *Antimonpräparate* ausfindig zu machen, was nach anfänglich vergeblichen Versuchen dann in enger Zusammenarbeit von Heilversuch und chemischer Synthese—mit Hans Schmidt sowie von Kuhn u.a.—gelang.

Diese Präparate (Stibenyl, Stibosan, Neostibosan, Antimosan, Neoantimosan u.a.), deren Wirksamkeit von uns im *Tierversuch* bei Nagana, Hühnerspirillose, Recurrens, Kaninchensyphilis, Kalaazar (Hamster), Bartonellenanämie der Ratte und Vogelmalaria ausgewertet wurden, haben sich auch besonders bei menschlichen Tropenkrankheiten zum Teil als sehr wirksam erwiesen. Vor allem haben diese organischen Antimonpräparate die üblen Nebenwirkungen des bisher angewandten Brechsweinsteins verloren. Sie zeigen eine viel grössere Wirksamkeit, wodurch eine *Massenbehandlung* tropischer Volksseuchen erst ermöglicht wurde. Interessant ist die immer wieder beobachtete *Spezifizierung* und *Spitzenwirkung* der verschiedenen Antimonpräparate. Das gilt besonders für die Behandlung der in Indien (China, usw.) verheerend auftretenden Kalaazar, mit Stibosan (693) und vor allem mit *Neostibosan*. Denn selbst die schwersten Fälle von Kalaazar, die sonst fast immer tötlich verliefen, konnten mit 8 intramuskulären (resp. intravenösen) Injektionen (2, 3 g.) Neostibosan in 8-10 Tagen in 98 prozent geheilt werden (Napier), während eine Kur mit dem schlecht verträglichen und giftigen Brechweinstein 2-3 Monate dauerte und die Zahl der Todesfälle noch 12-25 prozent betrug. Ähnliches gilt auch für die übrigen Formen der Leishmaniosis (Orientbeule, Espundia). Der Bericht der Calcutta School of Tropical Medicine (R. Knowles) 1934, S. 29 kommt zu folgendem Urteil : " the treatment of kala-azar with the pentavalent antimony compounds to-day is much more satisfactory than the treatment of syphilis or of human trypanosomiasis with organic arsenical derivatives " . . . . " is one of the most notable advances made in tropical medicine during the present century." Nicht minder wertvoll haben sich das dreiwertige *Antimosan* und besonders das *Neoantimosan* (Fuadin) bei der Massenbehandlung der in den Tropen (Ägypten, Japan, usw.)

weitverbreiteten Bilharziosis (und anderer Wurmkrankheiten) erwiesen, bei der in Ägypten 97 prozent mikroskopisch erwiesene Heilungen festgestellt wurden (Khalil). Auch bei *Opisthorchis*, Lungen- und Leberegel-Infektion (Clonorchiasis) findet dieses Präparat erfolgreiche Anwendung. Ebenso haben sich beim venerischen Granulom besonders die 5-wertigen Antimonpräparate (Stibenyl, Stibosan, Neostibosan) ausgezezeichnet bewährt. In neuerer Zeit wurden von Uhlenhuth, Schmidt, Seiffert und Kikuth auch die *Kombinationspräparate* von Arsen und Antimon chemotherapeutisch geprüft. Dabei zeigte ganz besonders das Präparat 386 *B* bei der *Bartonellenanämie* der Ratte einen auffallend günstigen chemotherapeutischen Index von 1:3500 bis 3750, was aussichtsreiche Heilerfolge auch bei dem menschlichen Oroyafieber (Verruga peruviana) erhoff enlässt. Auch bei *Opisthorchis felineus*- Infektionen der Katzen sah man ausgezeichnete therapeutische Erfolge (Österlin) mit 386 *B*. Ebenso hat 386 *B* bei Orientbeule, Leishmaniosis usw. eine gute Wirksamkeit entfaltet (Mühlens), sodass auch dieses Kombinationspräparat einen beachtlichen Fortschritt darstellt. Weitere therapeutische Versuche mit organischen Antimonpräparaten in der Praxis sind notwendig.

Dr. F. Nitti and **Dr. D. Bovet** (Paris) : *La chimiothérapie antistreptococcique. Relations entre la structure moléculaire et le pouvoir protecteur.*

A la suite des travaux de Domagk sur la sulfamido chrysoïdine, Tréfouel, Nitti et Bovet ont pu démontrer que le *p*-amine-phenylsulfamide lui-même, possédait une action protectrice dans l'infection streptococcique expérimentale de la souris, la fonction azotique étant inutile sinon gênante : NH_2 SO_2NH_2 (1162 F.) Fourneau et ses collaborateurs ont montré en outre que l'action protectrice du *p*-amino-phenyl-sulfamide est étroitement liée à la structure moléculaire. Les deux isomères ortho et méta sont inactifs. De même l'adjonction d'une troisième fonction (NH_2) sur le noyau benzénique du *p*-amino-phenyl-sulfamide rend ce corps presque inactif. Le *p*-amino-phenyl-sulfamide est, chez l'animal, extrêmement peu toxique et agit par voie buccale et parentérale. Ce corps protège la souris et le lapin infectés avec des streptocoques hémolytiques très virulents, mais par contre son action est presque nulle vis-à-vis des souches de faible virulence.

Cette protection peut se limiter à un retard constant et marqué de la mort des animaux traités vis-à-vis des témoins ou être définitive pour au moins 50 pour cent des animaux en expérience.

Les animaux guéris ne sont pas immunisés contre une infection streptococcique ultérieure.

L'action protectrice du *p*-amino-phenyl-sulfamide a été confirmée en France par Goissedet, Despois, Gailliot et Mayer, et en Angleterre par Colebrook et Kenny et par Buttle, Gray et Stephenson.

Ces derniers auteurs ont montré en plus que ce corps protège la souris contre une infection méningococcique expérimentale particulièrement sévère.

Dr. H. Bloch-Michel, Dr. M. Conte and **Dr. P. Durel** (Paris) : *Traitement de* 180 *cas d'érysipèle par le Benzyl-amino-benzene-sulfamide.*

Comme MM. Nitti et Bovet l'ont démontré, les propriétés antistreptococciques des corps récemment découverts sont liées au groupement $H_2NO_2S\langle\ \rangle NR_2$. Pour contrôler ces travaux nous avons choisi comme maladie d'épreuve l'érysipèle ; l'expérimentation a été conduite dans le service du Professeur Lemierre et sous sa direction.

Nous avons pu vérifier l'activité dans l'érysipèle de l'amino-benzène-sulfamide, mais lui avons préféré pour l'utilisation humaine le benzyl-amino-benzène-sulfamide.

Nous avons traité 180 sujets avec des doses quotidiennes allant de 2 gr. à 2,50 gr. per os : sur ces 180 malades nous n'avons observé aucun signe d'intolérance. 35 de nos malades étaient entrés avec une fièvre dépassant 40° : pour 27 d'entre eux, la normale fut atteinte entre 24 à 48 heures ; 7 autres étaient à 37° le 3e jour ; 1 seul ne parut pas influencé.

La plaque érysipélateuse subit une évolution parallèle avec un retard d'environ deux jours sur la courbe thermique. Nous avons traité 9 érysipèles des membres ; la mortalité est de 19 à 20 pour cent ; nous avons eu la chance de ne perdre aucun de nos malades. Un autre malade atteint d'un très grave érysipèle de l'abdomen a pu guérir, vraisemblablement sous l'influence du traitement.

Il semble, d'ailleurs, que l'action vis à vis du streptocoque ne soit pas exclusive.

Dr. F. Hawking (Cardiff) : *Arsenic-resistance in Trypanosomes ; and its Relation to the Non-absorption of Arsenical Compounds.*

In experiments *in vitro* on *T. rhodesiense*, it was found that normal trypanosomes absorb typical trivalent arsenicals with great readiness, while atoxyl-resistant ones do so only to a much less extent. This difference of behaviour, however, is not absolute, but only quantitative, since resistant ones will begin to absorb if placed in much stronger concentrations. Dead resistant ones absorb it very readily. Arsenicals to which both strains are highly sensitive, e.g., phenyl-arsenoxide, are absorbed readily by both strains ; the pentavalent tryparsamide is not absorbed *in vitro* by either. Injury of the parasite is secondary to absorption and the insensitivity of resistant trypanosomes to the arsenical is due to its non-absorption (as believed by Ehrlich), and not conversely (as maintained by Reiner), since (1) normal trypanosomes may absorb arsenical, but

show no signs of injury even after 24 hours, (2) absorption occurs rapidly, but injury shows itself only after a latent period of hours. The presence and degree of resistance to pheynl-arsenical compounds is closely associated with the presence and nature of the side-chains attached to the phenyl ring; thus atoxyl-resistant trypanosomes are highly resistant to the compound with the glycine-amido-side-chain (reduced tryparsamide), much less resistant to that with the amino side-chain (reduced atoxyl), and not at all to that in which the side-chain is absent (phenylarsenoxide).

Prof. Warrington Yorke (Liverpool) also contributed to the Discussion.

Dr. Margarete Zuelzer (Berlin).

Dr. Singer stellte als Voraussetzung vor seine Versuche die Behauptung, dass lebende Bakterien negativ geladen seien, die Protozoen dagegen positive Ladung aufwiesen.

Nach den Ergebnissen meiner Untersuchungen[1] sind lebende Protozoen und Spirochaeten ganz ebenso wie Bakterien ***negativ*** geladen. In der gleichen Gruppe können jedoch sowohl die verschiedenen Stämme als auch Individuen des gleichen Stammes verschieden *stark* negativ geladen sein. Entladene Spirochaeten werden in der isoelektrischen Zone starr und unbeweglich; jedoch ist dieser Vorgang reversibel. Bei erneuter negativer Ladung werden die Spirochaeten wieder beweglich. Positiv geladene Spirochaeten dagegen sind tot und daher biologisch bedeutungslos.

Ich muss also den Angaben des Vortragenden in Bezug auf die elektrische Ladung der Protozoen widersprechen.

Derartige elektrophoretische Messungen müssen mit exakten, jederzeit das gleiche Resultat ergebenden Methoden ausgeführt werden. Die Organismen müssen in kolloidarmen, besser kolloidfreien Medien gemessen werden, wobei *p*H, Temperatur usw. sehr wesentlich sind. Wenn der Vortragende sagt, dass unter den gleichen Bedingungen mit der gleichen Apparatur wechselnd und beliebig positive oder negative Ladung der gemessenen Organismen festgestellt werden kann, so ist das ein Beweis dafür, dass die benutzte Apparatur nicht den Anforderungen entspricht, die für derartige Untersuchungen notwendig sind.

Bei meinen derartigen Messungen erwiesen sich lebende Protozoen ganz ebenso wie Spirochaeten und Bakterien qualitativ stets gleichartig als negativ geladen.

Prof. J. Rodhain (Antwerp).

Le Professeur Schlossberger dans son magistral exposé sur la chimiothérapie, a insisté sur l'importance du rôle joué par les moyens

[1] *Zbl. Bakt.*, Abt. I, *Orig.*, 1932, 126, 360, und *Ref.* Abt. I, 1933, 1 06.

de défense naturelle de l'organisme qui complète l'action directe souvent imparfaite de l'agent chimique. Il est des cas où cette intervention n'est pas nécessaire et est du reste hors cause. Lors que par exemple il s'agit de produits agissant sur certains helminthes.

Pour donner un exemple concret, je ne conçois pas que l'organisme humain intervienne dans la guérison de la schistosomiase par les antimoniaux autrement que pour la cicatrisation des lésions et la résorption des parasites. Ces derniers sont bien réellement tués par l'agent thérapeutique lui seul.

Prof. E. Singer (Prague).

Die Untersuchungen beziehen sich auf das elektrische Verhalten von Erregern und chemotherapeutischen Substanzen im Blut des infizierten Tieres. Unter diesen Bedingungen sind Erreger positiv, Chemotherapeutika negativ geladen. Jede Aenderung des Milieus bedingt Aenderung in der Ladung, insbesonders können Versuche mit Kulturtrypanosomen nicht als massgebend für das Verhalten *in vivo* angesehen werden.

Allgemein ausgedrückt können diejenigen Substanzen von den Erregern aufgenommen werden, die vom Retikuloendothel granulär gespeichert werden.

DISCUSSION *on* FACTORS WHICH INFLUENCE THE TRANSMISSION OF INFECTIONS BY ARTHROPOD VECTORS

Chairman : Prof. E. Brumpt (Paris).

Prof. E. Brumpt (Paris), **Opener** : *Facteurs qui agissent sur la transmission des infections par les arthropodes hématophages.*

Les facteurs qui agissent sur la transmission des infections par les Arthropodes vecteurs sont très nombreux. Afin de limiter cette revue, nous ne nous occuperons pas des Arthropodes hôtes intermédiaires des Helminthes malgré l'intérêt qu'en présente l'étude. Cette réserve faite, nous étudierons les conditions qui influent sur la transmission des virus cytotropes, de certaines bactéries, de plusieurs Protozoaires, et enfin de quelques Filaires.

D'une façon générale, nous pouvons envisager l'importance relative :

1° des germes (visibles ou non) ; de leur aptitude à évoluer, de leur résistance vitale.

2° de l'arthropode vecteur.

3° du vertébré porteur de germes.

4° des conditions climatiques et de leur action directe ou non sur les vecteurs et les germes.

5° des modifications de l'habitat et de l'abondance des vecteurs dues à l'action de l'Homme.

I. *Les Germes.*—Que les germes aient ou non un hôte vecteur, ils présentent dans la nature un degré spécial de résistance aux divers facteurs physico-chimiques, résistance (ou sensibilité) qui pourra se manifester de même dans le corps de l'arthropode.

D'une façon générale, on peut dire que l'aptitude à évoluer d'un germe dépend en partie de son origine (malade, région géographique) ; de sa généalogie chez les animaux inoculés et du nombre de passages directs effectués ; du moment dans l'infection auquel le germe est puisé par le vecteur.

Les facteurs qui déterminent la diminution de la capacité évolutive sont encore bien peu connus. On peut parmi eux citer l'abaissement de température : *Spirochæta gallinarum* chez *Argas persicus ;* virus de la dengue chez *Stegomyia fasciata.*

Contrairement à l'opinion de certains auteurs, il ne semble pas que le changement d'hôte vecteur puisse modifier la virulence et les autres propriétés biologiques d'une souche. Contre cette idée, en effet, parlent tous les faits d'observation (*Spirochæta duttoni* transmis au Sénégal par *Ornithodorus erraticus* et en Afrique équatoriale par *O. moubata*) et d'expérimentation (virus du typhus exanthématique ayant évolué chez des puces, de la fièvre pourprée transmise par des poux et ayant conservé la virulence initiale ; *Trypanosoma cruzi* évoluant chez des Réduvidés, des punaises ou des tiques sans variation de pathogénicité).

II. *L'Arthropode Vecteur.*—D'un point de vue général, on peut dire que l'efficacité d'un arthropode hématophage en tant que vecteur dépend des facteurs suivants :

1° l'espèce et la race à laquelle ils appartiennent.

2° leur abondance.

3° leur habitat et les rapports plus ou moins étroits qui s'établissent avec les hôtes réceptifs.

4° du degré de leur préférence pour le sang des hôtes réceptifs et accidentellement de l'existence chez eux du cannibalisme, de la coprophagie ou encore d'un mode d'accouplement favorisant la contagion entre conjoints.

5° de leur aptitude plus ou moins grande à atteindre les germes morbides à des stades différents de leur existence (larves, nymphes adultes).

6° de la concordance entre leur régime d'hématophagie et le cycle, chez le vertébré, des parasites qu'ils peuvent transmettre.

7° des hôtes réservoirs de virus qu'ils ont pu piquer avant de s'attaquer à l'Homme, et des animaux sur lesquels ils se sont nourris une fois infectés.

8° de la présence de germes susceptibles d'évoluer dans le sang de l'hôte porteur de virus.

9° de l'immunité conditionnelle de certains vecteurs.

10° de l'existence de souches parasitaires plus ou moins aptes à évoluer.

11° de leur aptitude à permettre une évolution complète d'un germe aboutissant à des formes métacycliques infectieuses.

12° de leur flore intestinale qui peut s'opposer au développement des germes.

13° de l'action plus ou moins irritante de leur salive, le prurit pouvant déterminer un grattage favorisant l'infection.

14° de l'action que leur salive peut exercer dans le cas de certaines microfilaires qu'un tropisme attire ainsi vers l'arthropode.

15° de l'action de la température sur leur digestion qui favorise la fréquence des repas et l'évolution métacyclique des germes.

16° du mécanisme suivant lequel ils transmettent ces germes.

17é de la durée du repas.

18° de la possibilité pour certains arthropodes vecteurs d'être ingérés par leurs hôtes.

19° de l'attraction plus ou moins grande qu'exercent sur le germe les organes de l'arthropode (glandes salivaires, ampoule rectale, tubes de Malpighi).

20° de la durée de leur pouvoir infectieux.

21° de l'absence d'immunité acquise après une première infection.

22° d'une grande résistance ou d'une tolérance, de l'arthropode au germe à transmettre.

Un fait dont il est bon d'être convaincu c'est qu'une espèce animale n'est hôte vecteur que parce qu'elle offre un milieu favorable au parasite et lui permet d'arriver soit passivement soit plus ou moins activement chez son hôte définitif. Le virus de la fièvre jaune par exemple peut se conserver chez un grand nombre de moustiques mais chez quelques-uns seulement il est attiré par les glandes salivaires, ce qui permet son inoculation facile. Mais l'inoculation par la salive n'est pas nécessaire dans beaucoup de cas : les parasites peuvent être évacués avec les déjections, libérés par l'écrasement du corps de l'arthropode ou même ingérés avec celui-ci. Ce sont les tropismes propres des parasites qui déterminent leur voie de sortie et l'efficacité de celle-ci ne se peut juger qu'aux résultats obtenus. Rien ne permet de penser que l'inoculation salivaire, pour plus favourable qu'elle paraisse soit plus parfaite qu'une autre ; elle ne peut être considérée ni comme une acquisition ancienne au point de vue de l'évolution ni comme une nécessité pour la conservation de l'espèce, et son absence chez un vecteur ne nous autorise pas, comme le font certains auteurs, à considérer celui-ci comme "adapté récemment."

III. *Vertébré Porteur de Germes.*—Dans certains cas particuliers le séjour prolongé chez le Vertébré peut produire chez le parasite des modifications biologiques ; c'est le cas des infections anciennes à Trypanosomes ou le Protozoaire perd son pouvoir d'évolution chez la Glossine.

IV. *Facteurs Climatiques.*—Les facteurs climatiques conditionnent certainement les maladies transmissibles du monde entier. Leur mode d'action est encore peu connu ; ils agissent probablement directement et indirectement sur les hôtes réceptifs, les germes, les arthropodes vecteurs et les ennemis de ces derniers.

La chaleur a une action sûre dans certains cas ; celle du froid a été mise en évidence à diverses reprises ; d'autres facteurs physico-chimiques entrent en jeu également : par exemple, l'action de l'humidité et de la sécheresse sur la marche des épidémies est bien connue.

V. *Facteurs Divers Déterminés par l'Homme.*—L'Homme peut avoir une grande influence sur la transmission des infections. On ne connait que trop le rôle favorisant sur les épidémies de certains facteurs sociaux : guerres, famines, etc. La mise en valeur agricole, l'éxécution de certains travaux peut modifier grandement une situation sanitaire soit en bien, soit parfois en mal. Si intéressante que soit à étudier l'action de l'homme, elle ne constitue qu'un des *éléments de l'interaction générale des êtres vivants.*

En résumé, si nous connaissons certains des facteurs qui agissent sur la transmission des parasites par les arthropodes nous en ignorons souvent encore le mécanisme qui tient en grande partie aux propriétés biologiques et en particulier aux tropismes des êtres vivants dont ceux qui nous intéressent ici ne constituent que des cas particuliers.

Prof. N. H. Swellengrebel (Amsterdam) : *Two Factors influencing the Transmission of* Plasmodium vivax *by* Anopheles atroparvus *in the Netherlands.*

These factors are "*healthy carriers*" and "*human habitations.*"

Healthy carriers.—In a village north of Amsterdam anopheline infection was most prevalent from the 16th of August onward, attaining its maximum in the second half of October.

There are three human sources of anopheline infection in that village : (1) "spring patients" (malaria before July 1st), (2) "autumnal patients" (malaria after July 1st), and (3) healthy carriers. As all anopheline infections occur after July 1st the question at issue is : By whom are anopheles infected, by autumnal patients or by healthy carriers ?

The answer is : Far more by healthy carriers than by autumnal patients, and for the following reason :

During the autumn of 1935 a group of 92 houses was kept under continual observation. The number of infected anopheles settled in these 92 houses was 1,329 ; the number of autumnal patients was 123, that of the healthy carriers 83.

These 92 houses fall into two groups : One group of 43 houses contained all of the 83 healthy carriers ; in the second group of 49 houses they were absent. The autumnal patients are fairly evenly distributed over these two groups. In the group with parasite carriers, 48 autumnal patients were living and 75 in the group without parasite carriers.

If the autumnal patients were the principal source of anopheline malaria, infected anopheles would be found in both groups of

houses in approximately equal numbers. If, however, the healthy carriers were the principal source of anopheline malaria, infected mosquitoes would be found nowhere but in the group of 43 houses.

We found neither of the two suppositions realized. But the second one very nearly hit the mark. Out of the 1,329 infected mosquitoes inhabiting the 92 houses during the autumn of 1935, 1,279 or 96 per cent. were found in the 43 houses with healthy carriers; the remaining 50 or 4 per cent. were staying in the 49 houses with a slightly larger portion of autumnal patients but without healthy carriers (see diagram).

This result shows the superiority of the healthy carriers as a source of anopheline infection over the actual malaria patients, under the conditions prevalent in that village, where a sick-aid fund provides medical attendance free of charge to all families, and where, consequently, parasites in the blood of malaria *patients* are quickly destroyed. Not so, however, in the blood of parasite carriers who believe themselves to be healthy and so neglect to visit their doctor, although it would not cost them a penny to do so.

The human habitation.—The parasites in the blood of these carriers are few in number. But anopheles taking their blood in autumn stand a fair chance of acquiring the infection because they have lost the capacity to mature their eggs and, consequently, the incentive to leave the house for the sake of oviposition. So they are always near the parasite carrier who, after often repeated feedings, finally succeeds in infecting them.

In this way such houses harbouring, on an average, 30 infected mosquitoes per season, become a focus of infection; not only to the other human inmates but also to the inhabitants of neighbouring houses, where some few infected anopheles are actually found.

In cases like this there can be no doubt that the spraying of such a centre of infection with suitable insecticides, for the sake of killing infected anopheles, is a true measure of malaria control.

But this holds true only under special conditions depending upon the biological characters of the anopheles concerned, viz., *Anopheles atroparvus*.

If *A. atroparvus* behaved like the far-eastern *A. maculatus* which never stays in a house but only enters it to take blood and to acquire or to transmit a malarial infection, spraying of houses with the object of killing infected anopheles would be useless.

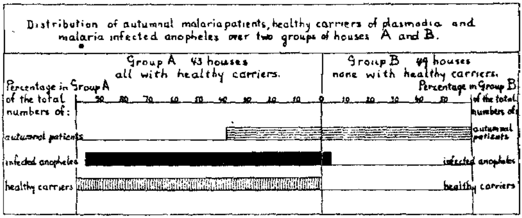
Conclusions.—I propose to distinguish between *house-born malaria*, i.e., malaria transmitted by anopheles which do not only visit the house but stay there quasi-permanently after becoming infected, and *field-born malaria*, i.e., malaria transmitted by anopheles which never stay in a house for any length of time.

In house-born malaria spraying is useful, but in field-born malaria it is not.

Anopheles causing house-born malaria can infect themselves on a healthy carrier because they *share* the house with him and so they can repeat indefinitely their infecting meals.

Anopheles causing field-born malaria require a much more potent parasite reservoir than the average healthy carrier, for they never really share a house with him. As a consequence they rarely have the opportunity to bite him more than once and so these anopheles cannot meet the principal requirement to become infected by a healthy carrier, viz., often repeated feedings.

Healthy carriers and human habitations may play an important part in malaria transmission. But they need not do so, and in the case they do, it is on the strength of the biological characters of the particular species of anopheles which acts as the local malaria vector.



Dr. H. L. Duke (London) : *Factors affecting the Cyclical Development of the Brucei-group of Mammalian Trypanosomes in* Glossina.

We may divide the summary into factors appertaining to the tsetse and those appertaining to the trypanosome. Of course, many factors act on both.

First of all, the tsetse factors : (1) The different species of tsetse differ in the readiness with which they transmit. Thus, *Glossina morsitans from N. Tanganyika is a better transmitter than G. palpalis* of Lake Victoria. In Nigeria, *G. tachinoides* is better than *G. submorsitans*.

(2) As regards temperature, Taylor in Nigeria showed that exposure of *G. tachinoides* to incubator temperatures during the period of the flies' infecting feeds raised the percentage of flies that acquired infection with *T. gambiense*.

My own experiments on similar lines with *G. palpalis* led me to conclude that the increased infection rate obtained by Taylor was due rather to the effect of the temperature on the metabolism of the fly than on the trypanosome direct.

Other observers, van Hoof and Roubaud, failed to demonstrate any increase in the infection rate in similar experiments. I believe that climate acts mainly in this indirect manner in influencing the infection rate in tsetse.

Yorke and his colleagues showed that in the developmental cycle of the *T. brucei* group in tsetse, infection of the fly's gut could take place at comparatively low temperatures, whereas the development in the salivary gland required a temperature of at least 75° F. (23.9° C.)

I have failed to demonstrate any differences between local races of *G. palpalis* in Lake Victoria in their power of transmitting trypanosomes.

Factors on the trypanosome side.—Experience has convinced me that the power of developing in tsetse (transmissibility) is a definite function of the trypanosome in nature, influenced but not dependent on external influences.

Some strains are more, some less transmissible and some again cannot develop in tsetse. Strains non-transmissible by *G. palpalis* have proved also non-transmissible by *G. morsitans*.

Prolonged sojourn in one and the same host, and prolonged passage from vertebrate to vertebrate by direct transmission tend, in my experience, to reduce the ability of a strain to develop in tsetse.

A trypanosome may be able readily to infect the alimentary canal of the fly but unable to establish itself in the salivary glands.

I have found *T. rhodesiense* generally better adapted to development in tsetse than the form of *T. gambiense* found in East and Central Africa.

A possible explanation of these observations is that *T. rhodesiense* is identical with *T. brucei*, a trypanosome admirably adapted to life in wild ruminants, whereas *T. gambiense* is in nature mainly dependent on man, a host to which it is far less biologically adapted than is *T. brucei* to game. The relatively intense reaction of its human host to the presence of *T. gambiense* renders the trypanosome unsuitable for development in flies.

The evidence accumulated in Uganda seems to confirm Robertson's views (1912) about the endogenous cycle of the trypanosome in the vertebrate. During certain phases of this cycle the trypanosomes in the peripheral blood are better able to develop in tsetse than in other phases. Of special interest is the occurrence of negative phases, during which the infectivity to fly is reduced or lost.

This theory explains the fact that the ingestion of large numbers of trypanosomes does not increase the likelihood of infection in the fly. On the contrary, the blood is often more infective to flies on days when very few trypanosomes are present.

My own work suggests that in certain species of vertebrate host the trypanosome is better able to avail itself of *Glossina* than in others. For example, in the guinea-pig *T. gambiense* tends to lose its transmissibility by tsetse, but transference of such an impaired strain to a *Cercopithecus* may restore its transmissibility. In certain species of antelope *T. rhodesiense* retains its transmissibility by tsetse for long periods.

When once a strain loses its transmissibility by tsetse the loss is, in my experience, permanent.

There appears to be some connection between the power of a trypanosome to develop in tsetse and its culturability in artificial media. Hitherto no one has succeeded in finding in tube cultures the infecting forms found in the salivary glands of the fly.

Occasionally infections die out in tsetse, either completely or from the gut only, the glands remaining infected.

The occurrence of double infections of two different species of trypanosomes in certain individual wild flies suggests that these are particularly suitable for the development of trypanosomes.

Repeated passage of one and the same strain in two parallel series through two different species of tsetse reveals no effect ascribable to the species of fly used. A single or repeated cyclical passages through tsetse do not increase the transmissibility of that strain by that species of tsetse, nor does cyclical passage affect such qualities as virulence to mammals or resistance—acquired or natural—to drugs or human serum.

***Dr. S. Adler** (Jerusalem): *Factors in Transmission of Leishmaniasis by Sandflies.*

THE FACTORS IN THE SANDFLY ARE :—(1) A sterile alimentary tract. Bacteriological sterility is essential for the continued development of *Leishmania* outside the body of the vertebrate host.

(2) Immunity of various species of sandflies to different strains of *Leishmania* and susceptibility to others, e.g., *Phlebotomus papatasii* is susceptible to *L. tropica* from Palestine, Algeria and Tunis, but refractory to *L. tropica* from Baghdad and Crete. The latter strains are transmitted by *P. sergenti*.

(3) The resistance in the sandfly is broken down by large doses of parasites.

(4) The immunity in the sandfly disappears entirely if it is fed on emulsions of parasites in saline or on emulsions of flagellates in liquid containing very little serum proteins.

(5) This immunity is not affected by washed red cells.

(6) The immune substances of the sandfly are either secreted by the stomach of the sandfly after feeding on serum, or are the products of digestion of serum.

(7) The above conclusions are made on the basis of observations in the field and on experiments with emulsions of flagellates made up with rabbit serum, red cells and saline.

FACTORS IN THE VERTEBRATE HOST.—These are not known in the case of *L. donovani*. Up to the present attempts to infect human beings by massive injections of culture of *L. donovani* and by L.D. bodies from the spleen and liver of hamsters have given negative results.

Probably the infectivity for the vertebrate host is maintained by cyclical development in the sandfly.

Prof. E. Hindle (Glasgow): *The Influence of Immune Substances present in the Blood upon the Transmission of Infections by Arthropod Vectors.*

Among the factors which influence the transmission of infections by Arthropod vectors little, if any, attention has been paid to the possible effects of the ingestion of antibodies together with the infective agent present in the blood.

In the case of infections due to filterable viruses, it is well known that in certain cases virus and antibody may exist together in the blood. An investigation of the period of infectivity of the blood in the case of rhesus monkeys infected with yellow fever, showed that these animals failed to infect mosquitoes fed on them in the last stages of their infection, although virus was still present in the circulation as detected by subinoculation. These results can be explained on the assumption that a certain degree of antibody titre prevents the virus from becoming established in the mosquito (Hindle, 1930). The observations of Hudson and Philip (1929), showed that the blood of a monkey experimentally infected with yellow fever by the bites of mosquitoes, contained virus from the same day it was bitten until its death eight days later. Whereas, on the first day the blood of this monkey was infective, as tested by subinoculations into normal monkeys, in doses of 0.5 c.c., a dose of 0.05 c.c. was negative, but on the last day of infection when it died, the larger dose was negative whilst the small dose produced infection. This result is doubtless due to the higher titre of antibody later in the infection producing a passive immunity in the animal inoculated with the larger dose of serum.

Different batches of *Aëdes ægypti* were fed on this monkey each day, but only those fed on the second to the fifth day of infection became infected. This can be explained by assuming that when the antibody titre of the blood rose above a certain level, the antibodies ingested together with the virus prevented the development, or persistence, of the virus in the mosquitoes.

Experimentally, it was found (Hindle, 1930) that when *Aëdes* were fed artificially on virus suspensions they readily became infected, but when fed on mixtures of virus and immune serum none of them became infected, in spite of the relatively enormous doses of virus ingested. Moreover, these mixtures, when inoculated subcutaneously into rhesus monkeys, produced yellow fever infection.

With reference to other infections direct experimental evidence is lacking, but, in the case of *Leishmania*, Dr. Adler has shown that the immunity of *Phlebotomus* to certain strains of the parasite depends on the presence of serum. In the absence of serum these particular strains of *Leishmania* are able to establish themselves in the gut of the sand-fly. This inhibitory effect of the serum may well be due to the presence of antibodies ingested together with the parasite.

The very small percentage of *Glossina* becoming infected after feeding on blood containing *Trypanosoma gambiense*, may also be explained by such a hypothesis, for it is obvious that in the case of chronic infections of this nature there must be immune bodies in the blood. It seems not unreasonable to assume that in the somewhat critical conditions of an organism trying to become established in its Arthropod vector, the presence or absence of such a factor may well decide the issue.

Moreover, in the case of yellow fever, immune bodies present in the serum have been shown to prevent infection in the mosquito, and therefore this factor must be added to the list of those which influence the transmission of infections by Arthropod Vectors.

Prof. J. Rodhain (Antwerp).

Le docteur Duke a rappelé que jusqu'à ce jour il n'avait pas été possible d'obtenir l'évolution chez les Glossines, de Trypanosomes de culture, en ce qui concerne *Trypanosoma brucei* et *T. gambiense* du moins. Je puis dire aujourdhui qu'il n'en est pas de même pour *T. congolense*. Au laboratoire de Léopoldville Brutsaerts en collaboration avec Van Hoof ont obtenu des cultures de *T. congolense*, qui absorbées par *Glossina palpalis* ont achevé chez celle-ci leur cycle évolutif particulier.

Pour répondre à une question du Professeur Reichenow je dirai que je ne suis pas certain que les cultures elles-mêmes injectées comme telles à l'animal, infectent ce dernier. La publication ultérieure du travail des auteurs belges précités nous fixera sur ce point.

Prof. E. Reichenow (Hamburg).

Es wäre von Interesse festzustellen, ob *Trypanosoma congolense* in der von Professor Rodhain erwähnten Kultur noch virulent für Säugetiere ist. Mit meiner Congolensekultur, die jetzt schon 2½ Jahre alt ist, gelingt die Infektion der Säugetiere nicht. Ich erhalte *in vitro*, ebenso wie bei meinen Gambiensekulturen, immer nur den Teil der Entwicklung, der sich im Darme der Glossine abspielt, und alle Versuche, durch Aenderung des Mediums auch den Abschluss der Entwicklung zu erzielen, der im Rüssel bezw. in den Speicheldrüsen erfolgt, sind bisher misslungen. Uebrigens ist *T. congolense* viel leichter zu züchten als *T. gambiense*, da der bei Gambiensestämmen früher oder später eintretende Verlust der Entwicklungsfähigkeit in der Glossine—und damit auch *in vitro*—bei Congolensestämmen nicht oder jedenfalls viel später erfolgt, denn mein Congolensestamm war bereits 9 Jahre alt, als ich ihn in Kultur nahm.

Dr. H. L. Duke (London), commenting on Prof. Rodhain's remarks on cultures, said that though interesting, the fact that culture forms of the pathogenic trypanosomes were infective to tsetse did

not possess much importance unless the infection in the fly extended to the salivary glands or proboscis of the fly.

Regarding Prof. Hindle's observations, it appeared to him unlikely that washing the infecting trypanosomes free from the host's serum would increase their infectivity to tsetse, because the trypanosomes taken up by the fly had already survived the immune bodies prepared by the host. Moreover, exposure for the 2–3 weeks after the infecting feeds to the blood of highly resistant animals such as *Varanus* and the baboon did not appear to interfere with the development of the trypanosomes within the tsetse.

According to Robertson's theory the forms destined for development in the tsetse were the short forms that survived in the mammal during periods of the cycle when the numbers of trypanosomes were greatly reduced.

Prof. E. Hindle (Glasgow), in reply to Dr. Duke, remarked that even if a parasite were unaffected by substances present in the blood of the host, it by no means followed that its developmental stages in the Arthropod vector would have a similar resistance. In the case of *Leishmania*, the flagellate stage that developed in cultures, or after ingestion by the appropriate species of *Phlebotomus*, was well-known to be adversely affected by various mammalian sera, including human, although the non-flagellate stage living inside the vertebrate host was presumably unaffected.

Lieut.-Col. H. E. Shortt (Madras) :

I hesitate to enter the discussion on trypanosomes and only do so on account of certain comparisons drawn between them and *Leishmania*. Dr. Duke's suggestion that the apparent effects of temperature in raising or lowering the percentage of infections in insects fed on infected blood are really due to changes in the metabolism of the *insect*, and not to effects on the protozoal parasite, are borne out in the case of *L. donovani* and *Phlebotomus argentipes*. Thus the infection rate in different batches of flies was found to vary between 1–2 per cent. and 30–50 per cent, in experiments where the temperatures were constant and the only variable was the altitude ; in one case 130 ft. and in the other 5,000 ft. above mean sea level.

L. donovani and *P. argentipes* also bear out the finding in the case of trypanosomes, that the percentage of flies infected when fed on the blood of patients, or experimental animals, is not necessarily in direct proportion to the numbers of parasites in the blood.

With regard to Dr. Hindle's remarks on the difference in titre of immune bodies in the serum of yellow fever cases at the commencement of symptoms and just before death, he has suggested that this is higher at the latter time and that this is the reason why a small injection of blood is more likely to provoke an infection in an experi-

mental animal than a large injection. It seems to me that the death of the patient is itself evidence that immunity response has failed and I would like to ask him if there is any evidence to show what the comparative titres of immune serum would be in the case of blood taken just before death and that of blood from recovered cases.

Dr. Adler in his summary noted that when *Phlebotomus* is fed on emulsions of flagellates in saline, immunity in the insect is entirely destroyed. This fact is not surprising and is in line with recent work on viruses in which it has been shown that, whereas fresh blood may not be infective, the same blood, if allowed to clot, and if the clot is thoroughly washed in saline, is capable of producing infections. The presumption is that the immune bodies in the serum are washed away by the saline and the virus is then free to produce its pathological effects.

Prof. W. A. P. Schüffner (Amsterdam).

Zur Frage der Wirkung des Immun-Serums auf das Gelbfieber wurde in Amsterdam der Zeitfactor systematisch untersucht. Gewöhnlich lässt man die Mischung 1/2 Stunde binden. Es zeigte sich aber, dass sich die Inactivierung (Abtötung ?) des Virus schon innerhalb etwa einer Minute vollzieht. Nur wenn man Virus und Immunserum in der Spritze rasch mengt und dann der Maus intracerebral einspritzt, kann man erwarten, dass noch lebendes Virus gegeben wird—die Maus stirbt an Encephalitis.

Prof. P. A. Buxton (London).

I desire to point out, in a few words, the importance of acquiring more information of a precise and quantitative sort about the conditions which determine the numbers of mosquitoes, etc., and the length of their life. Appropriate technique is now available. It is desirable that entomologists should give attention to the matter, and provide a solid body of data, acquired in the first instance from uninfected insects.

Prof. N. H. Swellengrebel (Amsterdam).

Prof. Buxton's remark is well illustrated by what I said earlier this morning, when I showed that in the transmission of malaria in Holland all hinges on the behaviour of *Anopheles atroparvus*. But I object to this behaviour being studied in the laboratory. It ought to be studied in the field, because the point at issue is not what the arthropod may be made to do in the laboratory, but what it actually *does* do in the field.

Dr. C. A. Hoare (London), said that the transmissibility of trypanosomes by tsetse-flies depended largely upon the reactions of the insect-vector. In the case of the crocodile-trypanosome

(*T. grayi*) he had demonstrated that, in spite of the scanty infection in the blood of the crocodile, practically 100 per cent. of the tsetse-flies fed on it became infected. However, the development of *T. grayi*, successfully started in the majority of the flies, did not continue in all of them, for in over 40 per cent. it ceased after the fifth day, only about 18 per cent. being capable of retaining a permanent infection which permitted the trypanosomes to establish themselves and to complete their evolution. From these facts it would appear that, on the one hand, *Glossina palpalis* was highly susceptible to infection with *T. grayi*, but a large proportion of the flies subsequently *acquired* an immunity to this trypanosome; and, on the other hand, in the same species of tsetse the majority of individuals were *naturally* immune to *T. gambiense*, since only a small percentage of the flies became infected under the most favourable conditions.

Prof. R. T. Leiper (London), drew attention to the fact that the remarks of all the speakers had dealt solely with the transmission of Protozoal infections by Arthropods. He thought, therefore, that attention should be drawn to the need for further studies of the factors which influence transmission of helminth infections by Arthropods. He cited the rarity of *Filaria bancrofti* in the Egyptian Delta, although the highly efficient vector *Culex pipiens* was extremely common and Filariasis was prevalent in a number of villages on the edge of the desert. Similarly, in the villages in Ceylon the distribution of Filariasis and Elephantiasis was curiously uneven, although *Culex fatigans* was exceedingly prevalent.

Dr. Margarete Zuelzer (Berlin).

Bei einer ausgedehnten Epidemie von Hühnerspirochaetose im Gebiet der Ostsee konnte ich *Culex* als Überträger von *Spirochæta gallinarum* nachweisen.[1]

Argas persicus war in den Ställen der kranken Hühner nicht zu finden. Nachdem ich in *Culex* aus den Hühnerställen mittels Praecipitation Hühnerblut hatte nachweisen können, machte ich Übertragungsversuche mit *Culex*. Bei diesen konnte ich bis 30 Tage nach dem Saugen am Huhn die Krankheit mit *Culex* auf gesunde Hühner übertragen; demnach muss *Culex* als echter Überträger von *Spirochæta gallinarum* angesehen werden. Bei der *S. gallinarum*, welche durch *Culex* übertragen wird, handelt es sich nicht um eine besondere Rasse, denn experimentell gelang es, die gleichen Spirochaeten ebenfalls durch *Argas persicus* zu übertragen.

Die kranken Hühner waren teilweise stark von Milben befallen. Übertragungen mit *Dermanyssus* gelangen ebenfalls, jedoch nur höchstens bis 48 Stunden, nachdem die Milben am kranken Huhn gesogen hatten. Dabei handelt es sich also um eine mechanische Überbringung der Spirochaeten, während *Culex*, auf Grund meiner

[1] *J. Trop. Med. and Hyg.*, 1936, 39, No. 17

Versuche, ebenso wie *Argas persicus* als echter Überträger der Hühnerspirochaetose angesehen werden muss.

Dr. Muriel Robertson (London), **Prof. Warrington Yorke** (Liverpool), and **Dr. C. M. Wenyon** (London), also took part in the Discussion.

DISCUSSION *on* THE PARASITIC NEMATODES OF PLANTS

Chairman: Prof. E. Brumpt (Paris).

Dr. P. Bovien (Copenhagen), **Opener.**

In the recent literature we find descriptions of several new nematodes which are biologically more or less closely associated with plants as parasites, semi-parasites and saprozoa. Comparative studies on these relations are of the greatest interest for the parasitologist, but it still holds true that the genuine plant-parasitic species are exclusively found within the family Anguillulinidæ, which harbours free-living, saprozoic and plant-parasitic species, and, what is highly interesting, numerous species parasitic in the body cavity of insects. Steiner, Thorne, Goodey, etc., have described new and remarkable species of *Anguillulina* and of some other genera of the same family, which are interesting from the standpoint of histopathology (gall-formation, etc.). *Anguillulina aberrans*, described by Thorne, exhibits characters indicating relationship to both *Heterodera* and *Tylenchulus*. The gradual swelling of the gravid female is thus found in insect parasites as well as in the three plant parasitic genera mentioned. Here is a vast field for future research, in which intensive collaboration (exchange of slides, etc.) between phytopathologists and expert nematologists is highly desirable in order to escape too much confusion in the taxonomy of the difficult groups.

The attention of the phytopathologist is, however, mainly directed towards the species *Anguillulina dipsaci*, *Heterodera marioni* and *H. schachtii*, together with *Aphelenchoides*. It is his aim to try to find effective measures against these threatening pests. What have we reached as yet?

Application of chemicals can seldom be practised under open-air conditions, but in Denmark we have successfully controlled *Heterodera marioni* on tomatoes in hot-houses by means of soil treatment with a mixture of carbon disulphide and cresylated soap (*Chr. Stapel*). The method is uow widely used and appreciated by the growers. Triffitt and Goffart have continued the work of Baunacke, Rentsch, Molz, etc., and obtained highly interesting results, which may turn out to be of practical use in future. More work concerning the effect of root-secretions and the influence of various crop rotations is highly desirable. As to the cultivated plants, we shall

hardly be able to find varieties of strains which are *totally* immune to the attack of the nematodes mentioned, but in some cases we find a marked variation in susceptibility. Nilsson Ehle found that the Rex-barley was only slightly attacked by *Heterodera*. Further experiments (Jørgensen, Thomsen, Bovien) have confirmed this: the plants are attacked, but always less than other strains of barley. Some Danish strains of red clover are more resistant to the attack of *Anguillulina dipsaci* than the foreign strains. White clover is heavily attacked in Denmark, but it is evident that wild English strains as well as white clover from uncultivated pastures are much more resistant than the refined strains hitherto used. More work along this line may yield useful results.

The question of host-relationships and the existence of biological races has been dealt with by several authors (Steiner, *v.* Slogteren, Quanjer, Goodey, de Bruin Ouboter, Schmidt, Hornburg, Goffart, etc.), but the views are conflicting, and we are still waiting for the worker trained in genetics to discover the methods needed to bring light in the darkness.

Dr. T. Goodey (St. Albans, England): *Lesions caused by Plant-parasitic Nematodes of the Genus* Anguillulina.

For convenience of consideration such lesions may be broadly grouped under two main heads:—1. HYPERTROPHIC and 2. NON-HYPERTROPHIC.

(1) HYPERTROPHIC. 1. *Flower galls* due to (i) *Anguillulina tritici* in wheat causing great modification of floral tissue with atrophy of sexual organs; (ii) *A. agrostis* in the grass, *Agrostis alba*, with atrophy of floral sexual organs and great stimulation of glumes and pales. 2. *Leaf galls* on grasses due to (i) *A. cecidoplastes* on *Andropogon pertusus* Willd.: here the gall has a complex structure, with an inner nutrient zone of thin-walled cells and an outer thickened zone; (ii) *A. graminophila* on *Agrostis vulgaris*, and (iii) *A. graminis* on Fescue grasses: in both the mesophyll cells multiply and enlarge, irregular intercellular cavities are formed and vascular elements enlarge; (iv) *A. dipsaci* on numerous hosts causes not only local galls but generalized symptoms with swelling of affected areas and stunting of shoots: cells hypertrophy, become separated and vascular elements enlarge. 3. *Root galls* due to *A. radicicola* on cereals and grasses show multiplication and enlargement of cortical tissues and endodermis, formation of cavities in cortex and increase in size of vascular elements.

(2) NON-HYPERTROPHIC. Lesions in roots due to *A. similis, A. pratensis* and *A. bradys* show breaking-down of cell-walls, destruction of cell-contents, formation of cavities and discoloration of tissues. It is suggested that mechanical injury by the parasite will not account for the types of lesion cited, but that these represent different responses by the host to irritant secretions poured out by

the parasite. In hypertrophic lesions we have chiefly the presence of growth-stimulating and absence of tissue-destroying substances, whilst in non-hypertrophic lesions a general absence of growth-stimulating and presence of tissue-destroying substances.

Dr. Marjorie J. Triffitt (St. Albans, England) : *Methods of Dispersal of Plant-parasitic Nematodes.*

The rapid spread of plant diseases, due to parasitic nematodes, and the extreme difficulty of eradicating these pests, has emphasized the need for a wider understanding of the methods by which dispersal takes place. Intimately bound up with the problems relating to dispersal are the longevity and resistance to adverse conditions shown by the nematodes, and their powers of specialization for limited host groups and subsequent re-adaptation under special conditions.

It has been repeatedly shown that the stem-eelworm *Anguillulina dipsaci*, of which the infective larva forms the resting stage, may be carried actually within or attached to the outer coats of the seed of the host plant. This occurs in the case of certain weeds as well as in cultivated hosts and thus a natural means of dispersal is provided. It is possible that, by acting as reservoir hosts these weeds may foster an infection in soil for several years following a cultivated infected crop and during this period contaminated soil may be removed by the agency of seed potatoes and thus transmit infection to fresh areas. By this means strains of the parasite specialized for hosts other than the potato may be dispersed by the agency of seed potatoes.

This transmission by seed potatoes of strains specialized for other host groups has recently been found to occur in the case of *Heterodera schachtii*, oat strain cysts having been found in considerable numbers in earth adhering to tubers. The strain attacking beet may also be carried in this way, as well as in soil particles amongst the actual seed. Recent discoveries of natural infections of *H. schachtii* on wild hosts indicate that this parasite has a wide natural distribution, hence its appearance in a new area as a parasite of a cultivated host may in some cases be due to the presence of a pre-existent natural infection on a wild host.

Prof. R. T. Leiper (London) enquired if the lesions classified by Dr. Goodey as " hypertrophic " and " non-hypertrophic " were specific to the individual parasites or if the reaction depended on the host species or host tissue attacked. He cited instances in which the same species, or closely allied species, of helminths produced hypertrophic reactions in one animal host and non-hypertrophic in another.

Dr. H. R. Briton-Jones (Trinidad, W. Indies).

The previous speakers on this interesting subject have raised the question of the multiplicity of host plants of certain species of parasitic nematodes. This possibility has occurred to me in the case of *Aphelenchus cocophilus* which causes Red Ring disease of coconut palms in the West Indies.

Red Ring disease is very much more serious on palms which are being grown on cleared land or on land covered with secondary bush. In the past large areas of coconuts have been established on sugar estates whereby sugarcane is grown as a catch crop for a number of years up to eight after the coconut palms have been planted. During these years the soil under the sugarcane is tilled and in most cases manured. Later the growing of the sugarcane is discontinued and the land left to the main crop—coconuts. Under such conditions Red Ring disease causes so few deaths of palms that it ceases to be of much economic importance. The contrast between the amount of Red Ring disease in coconut palms established in sugarcane and those established in secondary bush or uncultivated land is most marked and suggests lines of work including a search for an alternative host plant amongst the weeds.

If land is regularly tilled in Trinidad the rank grasses and coarse woody shrubs characteristic of secondary bush are frequently replaced by softer weeds such as *Alternanthera* sp. and *Commelina* sp. It is not inconceivable that in the changing of the flora an alternative host plant for the eelworm *Aphelenchus cocophilus* is eliminated, hence the marked control effect of tillage and manuring where palms are established in sugarcane. This aspect of the problem will be investigated in the near future.

Dr. T. Goodey (St. Albans, England).

Prof. Leiper had asked whether amongst parasitic species of *Anguillulina* there was any evidence that a given species might provoke a hypertrophic reaction in one host and a non-hypertrophic reaction in another. In reply, Dr. Goodey mentioned the action of *Anguillulina dipsaci* which, though mainly provoking hypertrophic reactions, apparently does not cause swelling in attacks on potato tubers and sometimes merely causes discoloration in parasitized leaves. Referring to the need of co-operation between the plant breeder and the nematologist raised by Dr. Bovien, Dr. Goodey pointed out that, as a result of work being carried out at the Institute of Agricultural Parasitology, two varieties of oats have been found highly resistant to attack by *A. dipsaci* causing " tulip root " in oats. He also mentioned field observations on the rôle of weeds as reservoir hosts recently brought to his notice by a correspondent, who had found a common weed parasitized by *A. dipsaci* amongst oats suffering from " tulip root." Replying to a question asked by Dr. Briton-Jones about banana roots attacked by nematodes,

Dr. Goodey pointed out that his paper had dealt only with members of the genus *Anguillulina* whereas what Dr. Briton-Jones possibly had in mind was attack by *Heterodera marioni.*

Dr. P. Bovien (Copenhagen) also took part in the Discussion.

DISCUSSION *on* TYPHUS FEVER AND THE RICKETTSIAS

Chairman : Prof. E. Brumpt (Paris).

Prof. H. Zinsser (Boston, U.S.A.), **Opener.**

I confine myself to the typhus fevers proper, omitting closely related but immunologically separable members of Rickettsiæ diseases.

We recognize two established varieties : (*a*) Classical typhus of Europe, (*b*) Murine, " Tarbardillo," Old and New World Type.

Differentiation was recognized (1928) when Mooser studied scrotal lesions of guinea-pigs described by Neill (1917). Mooser recognized the differential importance of the lesions and described Rickettsiæ in tunica cells. The two varieties are closely related but differentiable and biologically fixed.

Similarities between them are : (1) Animals convalescent from one are immune to the other ; (2) Both give Weil-Felix reactions ; (3) Serological tests with Rickettsiæ give overlapping agglutinations ; (4) Killed Mexican Rickettsiæ vaccines protect homologously and partially against the European ; (5) Immune murine horse serum agglutinates Weigl vaccines ; (6) Such horse serum protects homologously against the European under proper experimental conditions ; (7) Characteristic brain lesions occur in both. Scarcity of these lesions in the murine is due to rapid evolution and accelerated immunization.

Differences between the two are : (1) Murine type in male guinea-pigs causes reactions in the tunica vaginalis (4th to 6th day) ; (2) Smears invariably show Rickettsiæ ; (3) in European type animals, transitory, scrotal swellings may appear in about 20 per cent. Rickettsiæ are scarce.

In respective types, temperature reactions in guinea-pigs show characteristic differences. Murine type is more virulent for rats and survives longer in rats than does the European type. We have never been able to obtain in rats damaged by benzol, X-ray radiation or other methods the accumulation of organisms which is routine with Mexican strains. Murine can be carried indefinitely in mice, whereas European dies before fourth or fifth passage.

It has been suggested these differences represent reversible adaptations. Our experience indicates that while it is possible to achieve temporary modification of one type in the direction of the

other, eventually each will return to its original form. Occasional strains of murine isolated from Mexican epidemics have temporarily behaved like European, but always revert to type in guinea-pigs or rat passage. This suggests that European type may have evolved from murine by continuous louse-man-louse transmission during the periods between the XVth and XVIIIth centuries.

Likelihood of Rickettsia ætiology was established by Prowazek, Da Rocha-Lima, Otto, Arkwright, Bacot, Wolbach and others. We have added observations with washed Rickettsiæ from intraperitoneal exudates, serological and immunological evidence. Tissue culture studies have similar ætiological significance. We find no basis for theory that filterable phase of typhus virus still exists. No explanation at present accounts for Weil-Felix reaction. Rickettsiæ and the Proteus bacilli possess common antigenic factors, but association in a cyclic relationship is conjecture. Rickettsiæ appear the sole etiological factors ; two related but distinct varieties can be recognized : the *Rickettsia Prowazeki* and the murine, for which we propose name " *Rickettsia Mooseri*."

There is agreement on main epidemiological facts but differences of opinion on minor phases. Louse transmission was accepted as the sole dissemination until study of endemic cases in United States (Maxcy, 1926) suggested other insect vectors and rodent reservoirs. Dyer (1931) discovered virus in rat fleas in Baltimore. With Castaneda and Mooser we found virus in brains of rats trapped in Belem Prison in Mexico. Thus murine virus has its reservoir in rats. It is transmitted from rat to rat by two varieties of fleas and by rat-louse, and passes from rats to man by fleas. Fleas carry it for a month or longer and recover. Efforts to incriminate bed-bugs have failed. It has been suggested that murine typhus is *endemic and sporadic*, whereas an epidemic is always due to European virus.

We believe when murine flea-borne typhus causes scattered cases in communities not infested with lice it remains localized. When similar cases occur in louse-infected quarters, the man-louse-man cycle begins, as in the other ; epidemics follow. Conversely isolated cases of European typhus may occur in louse-free communities and remain localized. Endemic or epidemic characteristics depend upon circumstances and not upon the virus. In America a sporadic type of European typhus is known as Brill's Disease. We have isolated three strains of virus from Brill cases ; every one proved to be European virus. Study of 538 Brill cases occurring in New York and Boston (1910–1933) has convinced us that some recovered European cases may harbour the virus for many years. One factor in inter-epidemic conservation of this virus is human carrier.

Accumulations of murine Rickettsiæ can be obtained in rats in which resistance is reduced by X-ray. A radiated rat furnished 10 to 15 c.c. of Rickettsia suspension. Vaccines so prepared protect guinea pigs against the homologous strain. Human vaccinations

are being done in Mexico. Horse serum, which protects, is produced with these vaccines. Such serum has been used prophylactically by Varela and Bustamante. Otto has confirmed our serum experiments. Mexican vaccines so obtained influence European infections, but imperfectly. We have sought other methods of producing European vaccine. Weigl louse vaccine is probably effective, but does not lend itself to large-scale production. Immunization with desiccated virus shows that when such virus remains alive it produces disease. When killed, it no longer immunizes.

We turned to tissue culture. Kligler and Aschner and we ourselves have shown that formalinized European tissue cultures protect guinea-pigs. We have modified Nigg-Landsteiner method for application on a larger scale.

This method shows promise of practical solution of European vaccine production. We have studied sero-vaccination. A guinea-pig used for neutralization tests of virus with convalescent guinea-pig serum, later reinoculated, was found immune. We treated guinea-pigs with adjusted mixtures of European virus and protective sera, as follows: (1) Infectious blood plus convalescent guinea-pigs' serum, (2) Infectious blood plus antimurine horse serum, (3 and 4) Tissue culture virus with each kind of serum. Virus-serum mixtures, after standing, were injected intraperitoneally. It is necessary to use mixtures containing enough virus to produce vigorous typhus in controls.

Results were "complete" and "partial." In "complete" experiments, animals receiving the serum-virus mixtures remained normal, while controls reacted severely. Test animals proved immune when reinoculated a month later. In "partial" experiments, there was slight excess of virus over neutralization. Even when complete neutralization was not achieved, the severity of the infection was mitigated.

Guinea-pigs can be immunized against European virus with neutralized mixtures of living virus and protective serum. Such virus might convert animals into carriers. We have injected large quantities of brain and blood from sero-vaccinated animals into other guinea-pigs, which showed no reactions.

Summary

Relationship of two types of typhus virus has been described. Their biological attributes and the epidemiological principles have been discussed. Methods of vaccine production, active and passive immunization have been described.

Methods of practicable tissue culture vaccine production with European Rickettsias have been described and effectiveness of such vaccines in guinea-pigs demonstrated.

Sero-vaccination has been found effective in animals and is offered for human immunization in epidemics.

Dr. R. E. Dyer (Washington, D.C.): *Extension and Present Distribution of Rickettsia Diseases in the United States.*

Endemic typhus fever or flea-borne typhus has been recognized in the southern part of the United States about fifteen years, although it was probably present prior to that time. At first the disease, occurring principally in the seaports, has extended to the towns of the interior and in some sections has lost its urban character to some extent. The areas where typhus is occurring in the rural districts are chiefly those where peanuts are extensively grown, as in the southern part of Alabama and Georgia. The present distribution of the disease in the south-eastern and southern States includes the seaports of Maryland and Virginia and the seaports and many of the towns of the interior of the other southern States bordering on the Atlantic Ocean and the Gulf of Mexico.

Until 1930 there was a steady increase in the number of typhus cases reported each year. It is thought that this apparent increase can be accounted for by increased recognition of cases by physicians. Subsequent to that time there was a sharp definite increase in the reported cases which definitely indicates an extension of the disease. The factors considered in this extension are: first, a coincident increase in the cultivation of food crops in the southern States and second, the possibility that some one or more of the native species of rodents may be acting as a reservoir of the disease in addition to the known reservoir in the common rat.

With the extension of the disease no change (other than the development of rural cases as noted) has been observed in the chief epidemiological features—association with the handling of foodstuffs, absence of lice and the late summer and fall prevalence.

The second Rickettsia disease present in the United States, Rocky Mountain spotted fever, has been recognized in medical literature about forty years. There was a gradual extension of the area affected, from the original known foci in the northern Rocky Mountain States, which may be explained largely by increased recognition of the disease and by the ever-increasing white population. In addition it is thought that an actual dissemination of infected ticks, *Dermacentor andersoni*, also occurred until practically the entire area covered by this tick was included in the known spotted fever area.

In 1930 the disease was recognized in some of the States of the middle Atlantic coast and in subsequent years has been found in many other States outside the area of distribution of *Dermacentor andersoni*. The common tick of the eastern area is *Dermacentor variabilis*.

It is thought that the disease is more prevalent throughout the whole United States than the reported cases indicate with the exception of the New England States. In these extreme northeastern States no definite cases have as yet been recognized. It is

suggested that low prevalence of some natural vector which aids in the distribution of the disease in nature, as the rabbit tick, may prove to be the deciding factor in the apparent absence of the disease in New England.

Dr. A. Felix (London): *The Serology of the Typhus Group of Diseases and the Classification of the Corresponding Rickettseas.*

The "Typhus Group of Fevers," comprising a considerable number of different varieties of the disease, is now firmly established. This recent development is mainly due to the application of immunological methods to the study of these diseases.

The serology of the typhus group is based on the antigenic relationship between the viruses (*Rickettsias*) of the different varieties of the disease and corresponding serological varieties of *Proteus.* This relationship is most strikingly demonstrated by the close correlation between cross-immunity and agglutination reactions with various types of *Proteus*. Cross-immunity between different varieties of typhus virus is invariably accompanied by identity of the serum reactions to *Proteus X*, while failure to obtain cross-protection coincides with dissimilarity in agglutination reactions. The antigenic analysis of typhus viruses is best accomplished by the combination of cross-immunity experiments in the guinea-pig with tests for agglutinin production in the rabbit.

The intimate relationship between the viruses of the typhus group and *Proteus X* appears to be irreconcilable with the view, almost generally held for many years, that there is only a purely accidental' community of antigen. Although the experimental transformation of *Proteus X* into *Rickettsia* has not been accomplished, it seems to me that the hypothesis which postulates a genetic relationship between *Rickettsia* and *Proteus X*, is much more likely than the pure chance theory.

A simple scheme has been suggested for the classification of the Typhus Group. It is based on the antigenic type of the virus, on which immunity and immunity reactions depend, and thus follows a principle generally accepted in microbiology. The suggested scheme is a simple presentation of our present knowledge of these viruses, and provides an adequate framework for inclusion of results of future work.

***Prof. W. Barykine** (Moscow): *De l'infection exanthématique inapparente chez l'homme.*

A côté du typhus exanthématique cliniquement manifesté on constate l'infection exanthématique inapparente dans différents groupes d'hommes et notamment (1) chez les individus bien portants, qui n'ont jamais fait le typhus exanthématique, (2) chez ceux qui l'ont subi antérieurement, et enfin (3) chez les convalescents (l'état de prémunition d'après Conseil).

Le virus exanthématique, qui séjourne dans l'organisme de porteur peut s'atténuer. Par les passages consécutifs chez les cobayes il peut récupérer sa virulence.

Ainsi le fait de la conservation du virus exanthématique à l'état silencieux dans l'organisme de porteur ne doit pas être négligé au point de vue épidémiologique.

Il est bien probable que la forme inapparente de l'infection exanthématique chez l'homme est la seule, sous laquelle cette infection persiste hors des épidémies.

La lutte contre le typhus exanthématique pour être efficace doit comprendre non seulement les cas de l'infection cliniquement manifestés, mais aussi ceux de l'infection inapparente.

Les saisons libres des épidémies doivent être considérées comme les plus appropriées pour l'extermination radicale de l'infection exanthématique.

***Prof. W. Barykine, Dr. A. Kompaneez, Dr. E. Bauer** and **Dr. A. Botcharowa** (Moscow) : *Une nouvelle methode de cultiver le virus exanthématique.*

Les méthodes proposées jusqu'à présent pour cultiver le virus exanthématique ne peuvent donner que des quantités de virus insuffisantes pour les buts pratiques (vaccination, sérothérapie, etc.).

D'après les expériences des auteurs le virus exanthématique se développe bien facilement à 38·0-38·5° C. sur l'embryon de poule âgé de 2 à 4 jours. A cette date on injecte dans le jaune d'œuf incubé une petite quantité du matériel, contenant le virus exanthématique (cerveau de cobaye ou intestins de pou infecté du virus).

Le développement de la culture se réalise dans 6-7 jours à 33° C.

Le virus exanthématique se développe non seulement dans les tissus de l'embryon, mais aussi dans les membranes de l'œuf infecté.

On peut effectuer les repiquages des cultures. On n'y trouve pas les *Rickettsia prowazeki*, mais dans les cellules intestinales des poux infectés d'après la méthode de Weigl on constate le développement abondant de ces corpuscules.

L'infection expérimentale de cobayes provoquée par la culture des auteurs s'écoule sous les traits tout-à-fait caractéristiques pour le typhus exanthématique. Cette infection crée chez les cobayes une immunité bien solide contre le virus exanthématique de passage.

Au point de vue anatomo-pathologique l'infection se caractérise par la granulomatose de petits vaisseaux, propre au typhus exanthématique.

L'injection du cerveau de cobaye en état fébrile sous la peau du lapin donne dans 15-25 jours la réaction de Weil-Felix nettement positive.

Ainsi la méthode des auteurs permet de maintenir en culture le virus exanthématique avec toutes ses propriétés immunisantes et de le récolter en quantité tout-à-fait suffisante pour les buts pratiques.

Dr. J. W. Wolff (Amsterdam): *Some Remarks on Sumatran Mite Fever and Scrub Typhus.*

On the plantation area of the east coast of Sumatra cases of mite fever, scrub typhus (both of the OXK-type) and shop typhus regularly occur, the first two diseases being in the majority. As early as 1909 Schüffner described mite fever and pointed out its close similarity to the Japanese tsutsugamushi disease. Later work by Walch and Keukenschrijver has confirmed this. During the last five years, Dr. Kouwenaar and I have done some experimental work (animal infections) with the virus of mite fever and scrub typhus, the result of which has been published in a series of papers. I will only mention some of the main conclusions of our work.

1. A large series of animal infections (monkeys, guinea-pigs, rabbits and mice) and a study of the clinical and epidemiological data have led to the conclusion that *mite fever* (a variant of the Japanese tsutsugamushi disease) and *scrub typhus* are caused by the same virus, the only difference being the presence or absence of a primary ulcer in the skin of the patient. This difference may be explained by a difference in transmitting vector. In mite fever the infesting mite makes a virus depôt *in* the skin, whilst in scrub typhus the virus may be deposited *under* the skin owing to the longer mouth parts of the infesting insect (as yet unknown: tick ?).

2. Experiments on Japanese monkeys (*Macacus fuscatus*) have shown that the virus of mite fever is not identical with that of the Japanese tsutsugamushi.

3. I will draw attention to the fact that white mice are very sensitive to injections with OXK—rickettsia-virus (mite fever, scrub typhus) as was first shown by Dinger in Batavia, reacting much better (90 per cent. mortality) than guinea-pigs.

Dr. Paul Giroud (Paris): *Variations quantitatives des Rickettsies du typhus exanthématique du cobaye aux lieux d'inoculation: importance du facteur hormonal.*

Au cours de la conservation à Paris du virus murin mexicain de Zinsser (245 passages vaginales péritoine) et du virus historique de Ch. Nicolle (134 passages cerveau péritoine), nous avons cherché les variations quantitatives et locales des rickettsies.

Le virus de Zinsser, inoculé dans le péritoine du cobaye mâle par *passages vaginales peritoine*, provoque un exsudat abondant au niveau de la vaginale, où l'on constate des cellules de Mooser et de nombreuses rickettsies libres. Ce même virus transmis par *passages cerveau péritoine* ne provoque plus de périorchite, l'exsudat tend à disparaître, les rickettsies aussi. Très rapidement ce virus, conservé ainsi, peut être perdu. Nous avons aussi essayé des passages dans une cavité close, *de plèvres à plèvres*, mais sans aboutir à l'adaptation du virus à l'endothélium pleural. Il semblait qu'un tissu particulièrement réceptif était nécessaire pour une culture

abondante de rickettsies. Lors de *passages par voie péritonéale sur cobayes femelles*, nous avions constaté la petite quantité de rickettsies et de cellules de M. dans les exsudats. *Des cobayes femelles, greffés* dans la région rétro-hépatique avec des testicules de cobayes et infectés, présentent, sur le feuillet du péritoine pariétal en contact direct avec le testicule greffé, un exsudat comparable à celui qui recouvre les vaginales du cobaye mâle. Les cobayes mâles castrés, injectés tardivement, ne localisent plus le même exsudat au niveau du diverticule privé de testicule.

Des cobayes femelles infectés, traités par des injections quotidiennes péritonéales d'extrait orchitique, peuvent présenter un exsudat contenant une certaine quantité de cellules de M. et de rickettsies libres ; l'extrait hépatique n'a pas semblé avoir une action du même ordre. *Des cobayes mâles, traités avec l'extrait orchitique et infectés avec le virus historique de Ch. Nicolle*, présentent au niveau de la vaginale dans certains cas, des cellules de M. et des rickettsies. Des cobayes témoins mâles infectés de même par passages cerveau péritoine présentent de très rares rickettsies et cellules de M. D'autres témoins traités avec l'extrait globulaire de R. Legroux ne nous ont donné que quelques rickettsies. *Les cobayes mâles âgés de 10 à 15 jours* localisent au niveau de leurs vaginales un enduit abondant, mais les rickettsies et les cellules de M. ne sont jamais aussi nombreuses que chez les adultes. Chez le cobaye mâle adulte l'injection dans le péritoine, après l'inoculation infectante, de testostérone favorise pour un virus déficient la pullulation des rickettsies et des cellules de M.

Conclusions.—Ces constatations expliquent chez le cobaye pour une certain part les variations quantitatives des rickettsies aux lieux d'inoculation. L'inoculation locale d'extrait testiculaire ou d'hormone mâle provoque une augmentation locale des rickettsies. De plus le développement plus ou moins intense des rickettsies suivant les âges et les sexes chez le cobaye pourrait jusqu'à un certain point éclairer la bénignité chez l'indigène tunisien du typhus de l'enfant, la moindre gravité du typhus chez la femme, la plus grande fréquence chez celle-ci et chez l'enfant de maladies inapparentes.

Dr. Paul Giroud (Paris) : *Influence de la précarence C sur le comportement du virus typhique historique et sur la destinée de l'immunité acquise.*

De nombreux expérimentateurs ont montré l'importance de la carence C dans l'évolution de diverses infections. Zinsser et Ruiz Castaneda ont vu que les animaux scorbutiques, infectés de Typhus murin, présentent une pullulation considérable de rickettsies.

Nous avons constaté que le virus historique de Ch. Nicolle provoque parfois chez le cobaye des symptômes anormaux. Nous avons mis des cobayes mâles à des régimes de précarence C. Une

émulsion de cerveau infecté de virus historique, inoculée dans le péritoine provoque dans ces conditions une orchite clinique (15 fois sur 149), comparable à celle du typhus murin. Dans ces mêmes conditions, l'incubation de l'infection est modifiée et peut être réduite à trois et six jours au lieu de huit jours temps normal. L'orchite apparait du 5e au 8e jour et dure de 1 à 4 jours ; à l'autopsie, on constate un enduit analogue à celui constaté au cours du typhus murin. Les passages d'exsudat de vaginales, riches en rickettsies, n'ont permis de conserver cette souche avec orchite clinique que pendant 7 passages, puis l'orchite a disparu laissant seulement comme témoin de l'infection locale un exsudat.

Ces faits qui montrent les modifications de l'évolution d'un virus fixe au cours d'un régime pauvre en vitamine C, nous ont porté à rechercher ce que devenait l'immunité antityphique au cours de régimes pauvres en cette vitamine.

Cet essai a été réalisé avec des cobayes infectés par voie péritonéale avec l'exsudat vaginal très riche en rickettsies du virus murin de Zinsser ; leur infection typhique était passée depuis plusieurs mois. Nous avons mis deux lots de 15 cobayes chacun à deux régimes différents, l'un riche en vitamine C, l'autre très pauvre en cette même vitamine. Le premier lot recevait sous forme de verdure au moins 100 mg. d'acide ascorbique par jour. L'autre lot était mis au régime de carence C de L. Randoin, auquel on ajoutait environ 1 mg. d'acide ascorbique par jour. Ces animaux ont été infectés, lorsque pour les premiers le taux des surrénales en vitamine C atteignait 1 mg. 50 par gramme de tissu frais, pour les autre 0 mg. 11. Ils ont été inoculés par voie péritonéale avec une émulsion de cerveau (virus typhique historique). Cet essai nous a montré que les cobayes en carence ou au régime normal, se comportent de même envers une réinfection ; cependant la possibilité d'une infection inapparente devait être éliminée. Les cerveaux de tous ces animaux prélevés ont été inoculés à deux nouveaux lots et ceux-ci éprouvés avec un virus typhique au bout d'un mois. Cette dernière épreuve a montré que certains cobayes (3 sur 15) étaient vaccinés et que seuls étaient vaccinés ceux qui avaient reçu les cerveaux des animaux en carence. Si nous n'avions pu constater une faillite complète de l'immunité au cours de la carence C, du moins nous avions pu provoquer une baisse telle de l'immunité que certains animaux avaient présenté une infection inapparente. Ces faits nous permettent de concevoir l'influence des famines dans la dissémination, dans l'exaltation des virus typhiques et dans certaines faillites de l'immunité.

Dr. J. Laigret (Tunis) : *Vaccination contre le typhus exanthématique à l'aide d'un virus typhique murin enrobé et vivant.*

Les vaccins vivants sont aujourd'hui d'usage courant en Afrique du Nord pour l'immunisation contre le typhus exanthématique. G. Blanc emploie, à cet effect, le virus des rats de Casablanca,

qu'il atténue par la bile. En Tunisie et en Algérie, nous utilisons le virus typhique isolé des rats du port de Tunis par Hélène Sparrow ; nous le traitons suivant le procédé que nous avons mis en œuvre pour la préparation du vaccin de la fièvre jaune : dessiccation des cerveaux (de cobayes et de rats), broyage en phosphate de soude, enrobage double en jaune d'œuf et huile d'olives. Une dose, représentée par un centicube de suspension huileuse, correspond à 1/200ième de cerveau. Nos premiers essais comportaient deux inoculations : cerveau de cobaye puis, vingt jours plus tard, cerveau de rat. Nous ne faisons plus qu'une unique inoculation de cerveau de rat. Les réactions sont insignifiantes : fièvre très légère dans 2 pour cent des cas, 98 pour cent des sujets supportant le traitement sans le moindre incident. Jusqu'à présent, toutes les constatations sont en faveur de l'efficacité de la methode. Le nombre des vaccinés atteint cinq mille ; les applications continuent.

Dr. A. Felix (London) stated the following reasons for his view that the relationship between *Rickettsia* and *Proteus X* was not accidental or " heterogenetic," but that the two organisms were genetically related :

(1) A number of distinct serological varieties of typhus virus were specifically related to a number of corresponding antigenic varieties of *Proteus X*.

(2) The occurrence of *Proteus X* strains coincided with that of typhus fever.

(3) The close correlation between cross-immunity and agglutination reactions with various types of *Proteus X* strongly suggested that more than some trivial antigenic community was at play. It was known that the O antigen alone, which is common to *Rickettsia* and *Proteus X*, was incapable of inducing immunity to infection with typhus virus. The two organisms must therefore be further linked up by some other, yet unknown, factor or factors.

(4) The antigenic differences which so far have been disclosed between *Rickettsia* and *Proteus X* were not greater than those known to exist between a smooth and a rough variant, or between virulent typhoid bacilli, which contain Vi antigen, and the avirulent variant, which is devoid of this antigen.

Dr. P. Lépine (Paris), **Prof. R. Bruynoghe** (Louvain), **Dr. J. W. Wolff** (Amsterdam), **Prof. E. Brumpt** (Paris), and **Prof. H. Zinsser** (Boston, U.S.A.), also took part in the Discussion.

DISCUSSION *on* IMMUNITY AGAINST ANIMAL PARASITES

Chairman : Prof. F. Mesnil (Paris).

Dr. E. Sergent (Algiers), **Joint Opener** [Communicated by Prof. F. Mesnil] : *Immunité ou prémunition dans les maladies à hémocytozoaires (paludismes, piroplasmoses).*

On peut distinguer parmi les maladies infectieuses deux groupes suivant la sorte de résistance qu'une première atteinte confère contre des atteintes ultérieures.

Dans certaines maladies infectieuses, l'accès aigu critique de première invasion, s'il ne se termine pas par la mort, aboutit à une guérison complète, clinique et parasitaire, à laquelle succède l'état d'*immunité* : l'organisme " désinfecté " résiste aux *réinfections*. Exemple : la rougeole.

Dans d'autres maladies infectieuses, l'accès aigu critique de première invasion ne conduit pas à une guérison stérilisante ; ni le microbe ni son hôte ne sortent vainqueurs du conflit, ils survivent tous deux ; le microbe reste latent, l'organisme reprend l'apparence de la santé et l'infection qui couve empêche toute *surinfection* par un autre microbe de même espèce. Cette résistance de l'organisme infecté à toute surinfection constitue la *prémunition*. Elle est donc due à la préséance du microbe " premier occupant " sur tout microbe nouveau-venu.

Au cours de la symbiose du microbe et de son hôte, les réveils de virulence de la *primo-infection* provoquent les " rechutes," les surinfections ne provoquent que des accès avortés ou " accès de prémunis." Après la guérison parasitaire, la *réinfection* redevient possible, à la différence de ce qui se passe dans les maladies à immunité. Ex. : la syphilis.

L'étude qui suit a pour objet la prémunition dans les maladies dues à des hémocytozoaires pigmentés : *Plasmodium, Hæmoproteus*, ou non pigmentés : *Piroplasma, Theileria*. Les conclusions s'appliqueront aux maladies à prémunition dues à des bactéries ou à d'autres protozoaires.

Tout le monde est d'accord pour reconnaître que dans les maladies à hemocytozoaires la prémunition est prouvée. Il s'agit de savoir si, en plus de la prémunition, ces maladies peuvent comporter l'immunité vraie.

Les preuves de la prémunition sont les suivantes :

(*a*) L'existence de *très longues latences* est connue. Ex. dans le paludisme humain : latence de *Pl. vivax* atteignant 11 ans, latence de *Pl. præcox* 12 ans. Dans les piroplasmoses bovines, latences multiannuelles. Dans les les anaplasmoses, l'infection occulte persiste la vie entière.

(*b*) La *résistance aux surinfections* pendant l'infection latente est amplement prouvée : Paludismes. Piroplasmoses.

(c) Le *retour de la sensibilité* après la guérison parasitaire est démontré. Ex.: Paludisme des passereaux à *Pl. relictum*. Paludisme des pigeons à *Hæmoproteus columbæ*. Paludismes humains.

Donc, le fait de la prémunition dans ces maladies est acquis.

Il y a deux degrés dans la prémunition : la prémunition d'espèce ou *spécifique* et la prémunition de souche ou *raciale*.

La prémunition spécifique est très solide et permet l'application de l'épreuve de Laveran et Mesnil des inoculations croisées. Elle sépare nettement les uns des autres les trois parasites humains : *Pl. præcox, Pl. vivax, Pl. malariæ*. De même, le sous-genre *Piroplasma, sensu stricto*, et le sous-genre *Babesiella*.

La prémunition raciale permet de distinguer par exemple plusieurs souches au sein de l'espèce *Pl. vivax*, ou de l'espèce *Theileria dispar* de la theilériose bovine méditerranéenne.

Le problème pratique de la prémunition comporte deux questions : l'acquisition de la prémunition, son entretien.

(a) Le meilleur mode d'obtention de la prémunition consiste à atténuer le plus possible la *crise* qui suit la contamination et précède le stade d'équilibre. Si la crise est totalement supprimée, on a la prémunition d'emblée, solution idéale, où la phase métacritique prolonge de suite la phase procritique. C'est l'acclimatement sans risque. Dans les pays de paludisme endémique, on aide la population à acquérir la prémunition par une médication systématique (procédés de la " prophylaxie clinique ").

(b) La premunition une fois obtenue doit être tenue par de fréquentes et multiples réinoculations, sous peine de défaillir. Ex.: les épidémies de paludisme sont beaucoup moins violentes et graves dans les régions où les Anophèles piquent presque toute l'année que dans les régions où existe une pause annuelle ou multiannuelle de l'activité anophélienne.

Le fait de la prémunition dans les maladies à hémocytozoaires étant prouvé, il s'agit de savoir si l'immunité vraie stérilisante existe parfois, c'est-à-dire si l'organisme peut résister à une réinoculation après la guérison de son infection latente.

La question se ramène donc à celle du diagnostic de l'infection latente. Affaire de technique.

Pour déceler une infection latente, on dispose des moyens suivants :

(a) La rupture de l'équilibre en faveur du parasite, grâce à un artifice qui provoquera une rechute révélatrice. Ex. la splénectomie dans les piroplasmoses, les paludismes, etc.

(b) L'examen direct au microscope suffit parfois à révéler que les globules rouges sont porteurs de germes. Ex. *Theileria mutans*.

(*c*) L'ensemencement du sang. Ex. : *Leishmania du gecko.*

(*d*) L'inoculation du sang à un sujet neuf et sensible (Isodiagnostic). Ex. : paludisme des passereaux à *Plasmodium relictum.* Paludismes humains. Paludismes des singes. Piroplasmoses à *Piroplasma bigeminum* et à *Babesiella.*

(*e*) La succion du sang par l'insecte connu comme second hôte, à qui l'on fait piquer ensuite un sujet neuf et sensible. *Ex.* : les moustiques dans les plaudismes, les tiques dans les piroplasmoses.

Le fait de rendre manifeste une infection latente est un *fait positif.*

Le fait de ne pas découvrir une infection latente est un *fait négatif* qui n'a pas de valeur par lui-même.

Explications possibles des faits negatifs.—Dans l'interprétation des résultats de la recherche d'une infection latente, plusieurs causes d'erreur peuvent intervenir :

(*a*) Le fait que le matériel inoculé ne contient pas de virus n'implique pas son absence du reste du corps. Ex. : theilériose bovine méditerranéenne à *Theileria dispar* : le virus transmissible par transfusion (mérozoïtes), qui existe toujours dans le sang de la circulation périphérique au cours de l'accès aigu, ne s'y retrouve qu'exceptionnellement au cours de l'infection chronique, les agamontes restant localisés dans le tissu réticulo-endothélial.

(*b*) La quantité de matériel inoculé peut être insuffisante pour produire l'infection. Ex. : l'isodiagnostic appliqué au paludisme humain exigerait une saignée de plus d'un demi-litre. L'importance de la dose " minima " suffisante est montrée par le fait que dans les essais de paludothérapie des inoculations de sang ou des piqûres d'anophèles introduisant sûrement des germes vivants dans l'organisme restent souvent sans effet, et il faut parfois plusieurs réinoculations successives pour arriver à infecter le sujet. Il faut remarquer que les patients hospitalisés soumis à la paludothérapie sont placés dans des conditions spéciales de confort qui peuvent aider leur défense organique contre les contaminations.

(*c*) Les parasites peuvent exister à l'état vivant dans le sang inoculé, mais l'influence d'un médicament peut rendre les schizontes " inertes " ou les gamètes " infertiles," d'où insuccès.

(*d*) Les parasites peuvent exister vivants dans le sang, mais sous une forme qui n'est pas transmissible. *Theileria dispar* fournit un double exemple à ce sujet ; d'une part, les gamètes de *Theileria dispar* persistent seuls sans schizontes pendant des années dans le sang de la circulation périphérique. D'autre part, une souche de

Th. dispar transmise par transfusion de sang perd sa sexualité, elle ne produit que des schizontes et ne peut plus infecter les tiques. Dans le premier exemple, *Th. dispar* est incapable d'infecter le bovin, dans le deuxième exemple, il est incapable d'infecter les tiques.

(*e*) Il faut tenir compte de la possibilité d'une très *longue incubation* ou phase procritique, qui peut faire croire, à tort, à une absence de contamination. Ex. : longue incubation dans le paludisme humain contracté en hiver en Hollande, dans le paludisme des passereaux à *Pl. relictum ; accès retardés* dans la theilériose bovine méditerranéenne.

(*f*) Il faut tenir compte de la possibilité d'une *infection latente d'emblée* : phase procritique se prolongeant en phase métacritique sans aucune manifestation extérieure. Ex. : paludismes humains, aviaires, du singe ; piroplasmoses bovines, ovines, etc.

Dr. N. Hamilton Fairley (London), **Joint Opener** : *Serological Reactions in Somatic Helminthiasis.*

Precipitin and complement fixation antibody is bound up in the globulin fraction of the serum and has been demonstrated where parasites infect the bile ducts (fascioliasis), blood vessels (schistosomiasis), lymphatic system (filariasis), viscera (echinococcosis), muscles (trichiniasis), subcutaneous tissues (onchocerciasis) and brain (cysticercosis). Eosinophilia and dermal hypersensitiveness are associated with intestinal parasites, but circulating antibody is rarely demonstrable unless invasion of host tissue occurs at some stage of the life-cycle as in migration of ascaris larvæ. Antigens in use consist of cyst fluid or saline, alcoholic or other extracts of larval or adult stages of the parasites.

Precipitin Reaction.

Specific precipitin was first demonstrated in the serum of a patient with hydatid disease by Fleig and Lisbon (1907) and subsequently confirmed by Walsh, Chapman and Storey (1908). Since then similar reactions have been reported in other diseases, notably in trichiniasis by Backman (1928). The mode of preparation of such antigens and value of the tests in clinical diagnosis are reviewed.

Complement Fixation Reactions.

Hydatid.—Ghidini (1907) first utilized the Bordet-Gengou method in the diagnosis of hydatid cyst of the liver ; this was confirmed and extended by Weinberg (1909). The scolex factor in determining antigenic potency and the value of hydatid fluid and scolex antigens are considered. Analysis of results.

Schistosomiasis—Yoskimoto (1910) reported positive results in *S. japonicum*, utilizing alcoholic extracts of adult schistosomes as antigen. Fairley (1919) obtained potent antigen from the molluscan intermediary by extracting the livers of snails heavily infected with bilharzia cercariæ with absolute alcohol. The results obtained in experimentally and naturally infected animals and man are analyzed.

Filariasis.—Alcoholic extracts of dried, powdered *Dirofilaria immitis* afford a satisfactory antigen for *Loa loa* infestations, less so for *Filaria bancrofti*, oncocerciasis and *Dracunculus medinensis* (Fairley, 1931). Analysis of results.

Group Nature of Reaction.—Most of these reactions are essentially of group nature, specificity extending beyond the species to the genus or, even the family. Thus, hydatid fluid or scolex extract will react with *Tænia saginata* and *T. solium* antibody. In schistosomiasis cercarial antigen derived from *S. spindale* will react with sera derived from patients infected with the three species of human schistosome as well as with cattle schistosomes, *S. indicum*, *S. mattheei* and *S. bovis*.

Antibody.—In animals experimentally infected with schistosomiasis (*S. spindale*) antibody appears in from 7 to 10 days and persists throughout the course of the disease. Its time of disappearance after cure is still under investigation, but following tartar emetic treatment and the disappearance of ova and symptoms, positive reactions may persist for as long as 4 years. Similar findings are recorded in hydatid and trichiniasis.

Nature of Helminthic Antigen.—Owing to the lipoid content of these alcoholic extracts preliminary standardization in the presence of Wassermann positive sera is essential; in the final test the antigen has to be diluted sufficiently to work outside the zone of positive reactions given by syphilis and yaws sera.

Antigens used in the complement fixation test are thermostable, acetone insoluble and alcohol soluble. They cannot be filtered without loss of potency, but retain their titre for many years if stored on ice. Both chemical and biological tests have failed to reveal the presence of protein. The hapten is either a lipoid or some substance closely associated with lipoid, and the group nature of the reaction suggests some lipoid complex common to the genus. On injecting these extracts into animals, antibody may appear. The explanation probably lies in the hapten uniting with host protein to form a complete antigen.

Prof. J. Rodhain and **Dr. L. van den Berghe** (Antwerp) : *Contribution à l'étude des Plasmodiums des singes africains.*

Parmi les parasites de la Malaria des singes africains, la transmission expérimentale n'a été réalisée que pour trois plasmodiums : celui trouvé par Gonder et v. Berenberg-Gossler chez *Cercocebus*

fuliginosus d'abord, et celui rencontré par Anderson et Cowdry chez *Callithrix* sp. (*personata*) ensuite.

Ces deux plasmodiums, dont seul le premier a fait l'objet d'une description minutieuse, ont été considérés, par erreur, comme se rattachant à l'espèce *Plasmodium kochi*.

D'autre part, le parasite rencontré chez *Papio babouin* par Ionesco-Mihiesti, Cotta, Radecowicie et Badenski, qui s'est montré pathogène pour l'homme et le singe, et que ces auteurs rattachent à l'espèce *inui*, n'a pas été décrit jusqu'ici, et son identification doit rester en suspens.

Des circonstances heureuses nous ont permis de retrouver chez un *Cercocebus galeritus fuliginosus*, du Congo Belge, un plasmodium qui nous paraît identique à celui de *C. fuliginosus*.

Nous avons pu le transmettre à une femelle adulte de *Papio anubis;* à deux *Macacus rhesus* et à un *Papio jubilæus*. Ces animaux se sont infectés respectivement après 10, 5, 3 et 14 jours. La pathogénité du parasite est en réalité faible et n'a nécessité aucune intervention thérapeutique pour juguler l'infection.

L'inoculation à l'homme chez 3 sujets différents n'a pas été suivie d'infection et deux essais d'immunité croisée avec *Pl. knowlesi* ont donné des résultats négatifs.

Description.—Le parasite jeune montre de bonne heure une vacuole et possède une ou deux masses chromatiques dont exceptionnellement l'une est minuscule (accessory dot). Le pigment apparait dans les grands anneaux qui avec leur vacuole volumineuse occupent le tiers de l'érythrocyte. Les pseudopodes sont en général obtus.

La division aboutit à la formation de rosaces comportant en moyenne 12 merozoïtes.

La durée de la schizogonie est de 48 heures.

Les globules rouges parasités augmentent relativement peu de volume ; colorés au Wright ils montrent des granulations d'un type très irrégulier.

Les microgametocytes possèdent un noyau avec masse chromatique compacte, entouré d'un large halo rosé. Les macrogametocytes ont un noyau excentrique formé de grains chromatiques réunis en une masse ovalaire.

Ce plasmodium peut être distingué des autres espèces décrites ou signalées chez les singes d'Afrique. Par son pouvoir pathogène, la durée d'évolution de sa schizogonie, il correspond au plasmodium vu par Gonder et v. Berenberg, dont la morphologie répond dans ses grandes lignes à celle décrite ici.

Le séparant de *Plasmodium inui*, nous proposons de l'appeler *P. gonderi* sp. n.

Prof. Claus Schilling (Berlin) : *Immunität bei Malaria und ihre praktische Bedeutung.*

Dass sich im Verlaufe der chronischen Malariainfektion eine

Immunität entwickelt, ist nach den Untersuchungen von *James*, *Ciuca* u. a. sicher. Ob es sich aber dabei um eine sterilisierende Immunität handelt, steht nicht fest ; auch die Versuche von *Ciuca*, der durch Verimpfung selbst grosser Blutmengen von Malaria-Immunen auf Gesunde in etwa 60 prozent keine Infektionen erzielen konnte, sind nicht beweisend, weil er die " Gesunden " aus einem endemischen Malariagebiet entnehmen musste. Hier sind noch eingehende Untersuchungen nötig.

Wahrscheinlich handelt es sich bei den gegen Neuinfektionen Resistenten um eine labile Immunität, um eine Prämunition, bei welcher Parasiten noch lange nach ihrem Verschwinden aus dem peripheren Blute im Körper vorhanden sind. Diese labile Infektion wird aber in endemischen Malariagebieten unter grossen Verlusten durch Sterben und Siechtum vieler Kleinkinder erkauft. Es widerspricht den Grundgedanken der modernen Hygiene, der Natur einfach ihren Lauf zu lassen und die allmähliche Durchseuchung untätig abzuwarten. Wir fragen uns vielmehr, ob es nicht möglich ist, diese Verluste zu vermeiden und trotzdem eine Durchimmunisierung der bedrohten Bevölkerung zu erreichen. Es ist denkbar, dass eine allmählige Steigerung der Resistenz eintritt, wenn man bei Kleinkindern die Anfälle mit Arzneidosen behandelt, die zwar die akuten Erscheinungen beseitigen, aber nicht alle Plasmodien abtöten ; die sicher eintretenden Rückfälle müssten in der gleichen Weise behandelt werden, sodass keine chronische Anämie oder gar Kachexie entstände, die Kinder sich vielmehr normal entwickeln.

Dass die Chininbehandlung der inducierten Tertiana die Entwicklung einer Immunität nicht verhindert, geht aus Versuchen von *Ciuca* 1930 hervor : er injizierte 25 Paralytiker, deren erste Malariainfektion durch Chinin abgeheilt worden war, zum zweiten Male mit dem gleichen Stamme ; 8 von ihnen machten einen regulären Anfall durch, bei 9 traten nur Parasiten, aber kein Fieber auf, und 8=32 prozent blieben anfall- und parasitenfrei waren 17=68 prozent also klinisch immun. Die Auffassung, dass die Behandlung nicht bei der Entwicklung der Immunität interferiere, wird auch von *Ambialet* (Algier) und *Van Nitsen* (Katanga) geteilt und praktisch verwertet.

Andererseits sagt *James* 1931, dass nach seiner Erfahrung Immunität nur entstehe, wenn man jeden Anfall oder Rückfall für einige Zeit seinen Lauf nehmen lasse, ohne mit Chinin zu behandeln ; und *Ciuca* äussert sich 1935 ähnlich.

Angesichts dieser Unstimmigkeiten möchte Verf. Untersuchungen in endemischen Malariadistricten empfehlen : bei Kleinkindern ist der erste Anfall von Malaria so zu behandeln, dass die Parasiten aus dem Blute verschwinden ; die dazu nötige Menge von Chinin oder Atebrin wird leicht zu ermitteln sein. Sobald das Fieber aufgehört hat und das Blut parasitenfrei geworden ist, unterbleibt weitere Behandlung mit Specificis, doch kann die Rekonvalescenz durch allgemeine Roborantien (Vitamine, Leberthran u.ä.) gefördert werden. Der mit Sicherheit zu erwartende Rückfall ist ebenso zu behandeln.

Schon nach wenigen Tagen oder Wochen wird sich ergeben, ob die Kinder normal zunehmen und sich weiter zufriedenstellend entwickeln. Die Neuinfektionen der Neugeborenen werden eine Kontrolle für die Wahrscheinlichkeit der Superinfektionen bei den im Versuch befindlichen Kinder ergeben. Nimmt bei diesen die Zahl der Rückfälle und ihre Schwere allmählich ab, so wird man berechtigt sein eine langsam fortschreitende Immunisierung anzunehmen. Bleiben Rückfälle über längere Zeiträume aus, so könnten experimentelle Neuinfektionen in Betracht gezogen werden, um den Grad der erreichten Immunität zu prüfen. Dass sich die Kinder etwa zu Gametenträgern entwickeln, kann durch zwischengeschobene Plasmochingaben verhindert werden. Der grosse praktische Wert eines solchen Versuches in endemischem Gebiet für die Bekämpfung der Malaria dürfte einen solchen Versuch wohl rechtfertigen, zumal da er jederzeit, wenn sich ungünstige Resultate ergeben sollten, abgebrochen und durch die gewöhnliche Behandlung ersetzt werden kann. Zweck dieser Zeilen ist es, einen solchen Versuch auf geeignetem Terrain anzuregen.

***Prof. T. W. M. Cameron** (Montreal, Canada): *Some Considerations concerning the Resistance of the Vertebrate Host against Helminth Parasites.*

Owing to the fact that the "helminth" parasites are very complicated organisms belonging to several phyla, that their physiological processes within the host are absolutely unknown, and that none can complete its life-cycle within a single host, it is impossible to apply the usual bacteriological concept of "immunity" to the reactions of the host to their presence. The term *resistance* is suggested as a more satisfactory expression of what actually takes place. This resistance may be complete, absolute or partial, natural or acquired, or a combination of these alternatives. Incompatibility refers to the lack of environmental factors necessary for the normal metabolism of the worms; resistance implies a host reaction, humoral or otherwise, against the parasite. Complete resistance means that the worm is killed, absolute resistance means that it cannot reproduce successfully, while partial resistance permits of successful reproduction, but the worm is otherwise interfered with in size, etc. Incompatibility may be complete or incomplete.

Natural resistance is resistance shown in the complete absence of previous infection; it may be absolute or partial. Absolute resistance is most frequently shown against parasites of unrelated, and partial resistance against parasites of more closely related, groups of animals. Induced resistance is the result of previous infection or an antigen and may also be absolute or partial.

Present evidence suggests that the most important form of protection is Incompatibility and that worms fail to become or remain established mainly because of lack of essential environmental factors.

When resistance is shown, there is no reason to suppose that there are any essential differences in the underlying mechanism from that shown against any other foreign irritant. The main defence reactions appear to be connective tissue hyperplasia to isolate the parasite and/or repair the damage, eosinophile accumulation and production to act against " foreign proteins," and the production of " foreign-protein " antibodies with the accompanying phenomena of hypersensitiveness. There is evidence that all of these phenomena may be affected by diet.

The fibrous tissue reaction has been little studied, and there is an urgent need for more intensive research in comparative pathology, *e.g.*, the comparison of the reactions of a variety of hosts to a common stimulant. There is an equally urgent need for biochemical and physiological research on the parasites themselves. Until much more work is done on these lines, theories must be hypothetical and data unsatisfactory.

Resistance to helminthic disease is not necessarily the same thing as resistance to the presence of the helminths and is referred to as Tolerance; when the effects of the parasite cannot be overcome by the host reactions, a state of Intolerance results.

Dr. W. J. Penfold and **Dr. H. B. Penfold** (Melbourne, Australia) [Communicated by Dr. J. V. Duhig (Brisbane]: *Cysticercosis bovis and its Prevention.*

In Australia this disease has occurred in many oxen on sewage farms, and, despite the economic loss involved, the farms have been prevented from raising cattle for human consumption, because of the risk to man of infestation with *Tænia saginata*. However, this economic waste is quite unnecessary, for cattle can be readily immunized against the disease.

In addition to being free from living cysticerci, beef should, if possible, be free from their degenerated remains and from the points of scar tissue which are left when the bladder worms degenerate.

Method of Immunization.

Artificially to immunize cattle against *Cysticercus bovis* we have given them large doses (400,000) of fresh *Tænia saginata* eggs by mouth, in this way producing a primary infestation. When after waiting for 54 weeks to 23 months we have attempted to produce a secondary infestation by another large dose of fresh eggs, we have invariably failed, showing that the cattle were immune.

We have found further that immunity can likewise be produced after animals have been allowed to feed on a sewage farm and so contracted very light natural infestation.

Of 88 cattle bought on the open market in Melbourne, which we infected artifically, each one contracted the disease. In such cases innumerable cysts (*ca.* 30,000) developed. The bladder

worms die after some time, when the cysts degenerate, diminish in size, leaving finally small gritty residual masses, or, alternatively, points of scar tissue. These degenerated cysts we have never observed in the first three months after artificial infestation. Death of the worm, with degeneration of the cyst, may occur in the early stages of the disease, beef measles, but in this case the cyst is filled with a greenish pasty material. In heavily infested oxen we have never found a living bladder worm later than nine months after infestation of the ox. We believe, therefore, that the risk of infecting man does not exist for more than nine months after infestation, but we cannot give a precise date after which no degenerated cysts can be found in the meat. We have found a few such cysts in one ox two years and two months after the artificial infestation occurred, so that it seems that two and a half or three years might have to elapse after primary infestation before we could guarantee the absence of any sign of the previous infection in 100 per cent. of animals.

We have found that the eggs of the tapeworm tend to accumulate in the pasture round the sewage outlets. Consequently we would recommend to those who propose to immunize cattle naturally on sewage farms that they should depasture young calves in close proximity to the outlets: this would ensure an early infestation and, consequently, an early immunity. Subsequently such calves could be pastured anywhere on the sewage farms with impunity, but if such animals were not slaughtered until they were about three years of age, we are satisfied that they could not spread *Saginata* tæniasis.

This natural method of immunity would be quite effective and easily used in Syria and in certain parts of India and Africa, where the disease is very prevalent. It would not be very effective in those cities where there are very few inhabitants with *T. saginata*.

As to the minimum number of cysts which will produce immunity, we know that the naturally infested animals that have been depastured on the Melbourne sewage farm have acquired very complete immunity from only small infestations.

Dr. Gilruth examined ten oxen that had been on the farm for an average of nine months, and found on the average five cysts to the carcase, from which we conclude that a relatively small number of cysts would be sufficient to produce immunity.

Prof. E. Rodenwaldt (Heidelberg).

Im Lande der Karoobataks in Sumatra findet man Trichinose bei Schweinen, Hunden und Ratten. Niemals wurde aber Tr. beim Menschen beobachtet, obschon die Batakker das Schweine- u. Hundefleisch halb roh essen. Mit Teilen ihrer Fleischmahlzeit kann man Ratten leicht infizieren. Mit Antigen, bereitet nach den Vorschriften von Augustine, Theiler, McCoy, kann man bei

Schweinen selbst bei einer Verdünnung von 1 : 1000–1 : 10,000 schwache Infektionen nachweisen sowohl durch Dermoreaktion als durch Præcipitation (1 : 3000). Niemals wurde eine Reaktion beim Menschen gefunden.

Auch bei Sektionen wurde beim Menschen niemals Trichinose gefunden.

Da die Immunreaktion immer negativ ist, kann man auch nicht eine schwache Infektion im Kindesalter annehmen, die etwa Superinfektion stärkeren Grades verhindert hätte. Man muss daher zunächst an eine Standortvariante der *Trichinella* denken, die für den Menschen nicht infektiös ist, vielleicht auch an eine Rassenimmunität der Batakker.

Prof. Rodhain teile ich mit, dass Gonder und ich *Cercocebus fuliginosus* mit *Plasm. gonderi* leicht infizieren konnten; die Infektion blieb im Blut manifest über ½ Jahr, wenn man die Affen entmilzte.

Gegenüber Prof. Schilling wird auf die praktischen Erfolge der Mückenbekämpfung in Niederl. Indien hingewiesen. Senkung der Mortalität in Tandjong Priok von 120–150 pro mille auf 25–30 pro mille; dieses Ergebnis besteht jetzt schon 13 Jahre. Praktisch braucht man nicht mehr zu verlangen. Dergleichen Ergebnisse wurden in Niederl. Indien an vielen Orten erzielt.

Dr. Hamilton Fairley teile ich mit dass Dermoreaktion und Præcipitation mit Antigen aus *Filaria immitis* bei Trägern von *F. malayi* keine brauchbaren Ergebnisse lieferten.

Prof. M. Cluca (Bucarest).

D'accord avec les faits, apportés par le Col. James, nous croyons que l'immunité dans le paludisme expérimental n'est pas nécessairement liée à la prémunition. Le contrôle du parasitisme latent par transfusion de sang à de nouveaux patients, dont l'état mental exige la malariathérapie, a été la méthode employée. Ce contrôle est de beaucoup plus précis chez les patients à immunité acquise vis- à-vis du *Pl. knowlesi*; le sang de 3 malades immuns à la suite d'une première infection, suivie de guérison spontanée, n'a pas donné l'infection au *Macacus rhesus* même après une inoculation endoveineuse de 15 c.c. de sang.

Prof. C. Schilling (Berlin).

Spezifische Behandlung unter Schonung des Prozesses der Immunisierung wäre einfacher und billiger als Anophelesbekämpfung, macht diese aber nicht überflüssig.

Prof. R. T. Leiper (London), commenting on Dr. N. Hamilton Fairley's paper said that the hopes, which the early serological work had raised, that these reactions would prove of value in elucidating or confirming the zoological relationships of helminth parasites, had been weakened, rather than strengthened by recent

work. Microscopical examination was proving more reliable and more specific than serological methods of diagnosis. The positive reactions obtained by the use of *Dirofilaria immitis* antigen in *Loa, Onchocerca* and *Dracunculus* and its failure in *Filaria bancrofti* confirmed the systematic helminthologist in the view that this test was an unreliable guide.

Commenting on Prof. T. W. M. Cameron's communication, of which the printed abstract alone was available, Professor Leiper demurred from the suggestion that it was impossible to apply the usual bacteriological concept of "immunity" to the reactions of the host to helminth infections. He thought that until much more work is done, helminth theories must be unsatisfactory and data hypothetical.

Lieut.-Col. S. P. James (London) and **Dr. K. Meyer** (Berck-Plage), also took part in the Discussion.

MISCELLANEOUS PAPERS

Chairman : Prof. E. Brumpt (Paris).

***Dr. R. Deschiens** (Paris) : *Comportement des formes végétatives et des kystes de l'amibe dysentérique, dans certaines conditions naturelles, exterieures à leur hôte.*

Du point de vue biologique et épidémiologique, les éléments parasitaires sont appelés à rencontrer des milieux liquides, des milieux secs ou en voie de déshydratation et des milieux à humidité permanente (eaux usées, poussières, denrées alimentaires).

Ces différents milieux réagissent par : (*a*) leur concentration, (*b*) leur température, (*c*) leur viscosité, (*d*) leur réaction chimique, (*e*) leur composition chimique, (*f*) la flore qui s'y développe et les produits résultant de l'activité de cette flore.

Voici deux épisodes biologiques qui illustrent ce que ces actions physiques et chimiques peuvent avoir de complexe :

1°. Dans certaines eaux d'égoût, isotoniques ou légèrement hypo- ou hypertoniques, entre les températures de 0° et 43° C., à un *p*H situé entre 6·6 et 8·2, en présence de matières organiques brutes ou peu dégradées (eaux usées de marchés et d'abattoirs), les formes végétatives de l'amibe dysentérique peuvent se conserver vivantes de 2 à 15 jours ; entre 32° et 43°, dans ces mêmes conditions, elles peuvent se multiplier, cultiver et éventuellement s'enkyster.

Les expériences réalisées ont eu pour base des eaux d'égoût, issues des abattoirs de Levallois (Paris) et des eaux usées provenant des systèmes d'égoûts médiévaux de la ville indigène de Fez (Maroc).

2°. Des masses de dattes et des masses de figues, séchées, ont été contaminées avec des kystes d'amibes dysentériques. Sur ce milieu très hypertonique, les kystes ont résisté de 15 à 25 jours.

Il est donc possible que des produits alimentaires exotiques, souillés au cours de leur récolte ou de leurs manipulations, contaminent les sujets qui les consomment, à la colonie ou même dans la Métropole.

Une observation communiquée par M. le Professeur Chiray (Paris) relate l'apparition d'une dysenterie amibienne chez un Parisien sédentaire après ingestion de quantités massives de dattes importées directement de l'Afrique du Nord.

Parmi les circonstances très diverses de circulation des éléments parasitaires en dehors de l'hôte, ces 2 exemples précis montrent les voies imprévues mais logiques qu'empruntent certaines contaminations.

***Prof. L. Riedmüller** (Zürich) : *Zum kulturellen und biologischen Verhalten von* Trichomonas fœtus.

Trichomonas fœtus (=*Tritrichomonas fœtus*) ist ein beim seuchenhaften Frühabortus und der Sterilität des Rindes vorkommender, nicht selten auch sporadischen Abortus verursachender Flagellat. Bei der Kultivierung kamen wir von der Verwendung von Amoebennährböden ab und benützen seit Jahren Serumbouillon oder Serumagar in hoher Schicht (5–10 prozent Serumzusatz, gewöhnlicher Agar 1 prozentig, Stichkulturen). Einige Stämme wachsen so bereits in über 400 Passagen. Der dass Wachstum fördernde Anteil des Serum ist thermostabil. Sowohl in Serum- wie in Blutbouillon werden Haemolysine in geringer Menge gebildet. Gewaschenen Erythrozyten und dem Blutfarbstoff scheint keine Bedeutung für das Wachstum von *Trichomonas fœtus* zuzukommen. Das *p*H-Optimum liegt bei *p*H 6·5–7·5 (Wachstum bei *p*H 5·5–8·5). In Uebereinstimmung mit Cailleau fanden wir starke Säuerung des Nährbodens nach Wachstum in Glukose, Galaktose, Laktose, Lävulose, Maltose, Saccharose, Raffinose, Stärke und Dextrin, schwache Säurebildung in Inulin, keine dagegen in Arabinose, Xylose, Rhamnose, Glycerin, Erythrit, Mannit, Dulcit, Sorbit und Inosit. Zudem konnten wir noch in Glykogen stärkere Säurebildung feststellen. Nahezu parallel mit der Säuerung ging die Vermehrung. In Bezug auf die Sauerstoffverhältnisse besteht eine auffallende Anpassungsfähigkeit. Gemeinsam mit H. Spoerri ermittelten wir den Sauerstoffverbrauch manometrisch nach Warburg in Luft, reiner Sauerstoffatmosphäre, sowie die Atmungslähmung in einem Gemisch von 95 prozent Kohlenmonoxyd und 5 prozent Sauerstoff. Es zeigt sich, dass die Atmungsgrösse in 24–48 Stunden alten, gewaschenen und in steriler Serumbouillon aufgeschwemmten Kulturen etwa 4·3 cmm. O_2 pro cmm. Trichomonadendepot (entsprechend 0·12 mg. Trockensubstanz oder ca. 2 Millionen Trichomonaden pro cmm.) ausmacht. Bei 60-stündigen Kulturen betrug die Atmungsgrösse noch 2·8 ,bei 3 Tage alten 0·81 cmm. In der Kohlenoxydsauerstoffatmosphäre trat keine merkliche Veränderung der Atmungsgrösse ein. Hinsichtlich der Atmungs-

katalysatoren fanden wir in *Trichomonas fœtus*-Kulturen spektroskopisch weder Cytochrom noch andere Haemochromogene. Die makroskopische Indophenoloxydasereaktion (Nadireaktion) und die Peroxydasereaktion waren negativ. Katalase ist höchstens in Spuren vorhanden. Die Methylenblaureaktion im Thunbergröhrchen ergab rasche und vollständige Entfärbung.

Dr. H. Sommer, Prof. K. F. Meyer, Dr. W. F. Whedon and **Dr. C. A. Kofoid** (San Francisco): *Mussel Poisoning.*

History: Some 120 cases of acute neurotoxic poisoning after consumption of mussels were recorded in northern Europe during the past 100 years; 240 cases along the Pacific Coast of the American North-west.

Results of Present Investigation near San Francisco: (1) Periodic tests over a period of eight years show that the poison is present in the mussels most of the time in small quantities and at least once during the summer, for several days, in dangerous amounts. (2) The occurrence of the same poison in various amounts in different kinds of clams, at various distances from the ocean, as well as the distribution of the poison in a given mussel-bed point to the water of the open ocean as the carrier of the poison.

Source of Poison: (1) Plankton counts by W. F. Whedon, under direction of C. A. Kofoid, show that a Dinoflagellate, *Gonyaulax catenella* n. sp., occurred consistently during the poison seasons, the numbers running roughly parallel to the degree of toxicity in the mussels. (2) Mussels, held in the laboratory in filtered sea water, under various conditions, never increase in toxicity, but always lose their poison. Only when sea water, containing a large number of *Gonyaulax catenella*, was fed to the mussels did their toxicity increase, as much as twentyfold. (3) The shellfish poison could finally be extracted directly from the plankton. The number of *Gonyaulax* per poison unit is fairly constant, *i.e.*, *2,000 to 5,000.*

Mussel poisoning along the northern California Coast is due to a specific Dinoflagellate. Plankton feeders take up and store the poison with their food and may become dangerous for human consumption. The same or a similar cause may hold for the European outbreaks. In this case a micro-organism with a habitat in the estuaries and harbours must be presupposed. Analogies with other intoxications, especially "water-bloom poisoning" of domestic animals, caused by freshwater algæ, are apparent.

Mr. Ralph Brown (Dublin): *The Use of High Centrifugal Forces in the Study of Cellular Structure.*

Various attempts have been made to centrifuge the cytoplasmic components of the cell. Even with the high-power electric centrifuge the effects have been slight.

becomes apparent that it is often difficult to reduplicate experimentally manifestations of disease that develop under natural conditions. Different types of infection are recognized, one of which, in the absence of reinfection, is self-limited, and runs a brief course, but is not terminated by any protective reaction on the part of the host. It is possible to reinfect repeatedly the latter, although eventually protective reactions may prevent further reinfection. It is notable, however, that species which regularly produce self-limited infection occasionally, in the absence of reinfection, continue to multiply until the host is overwhelmed. The conditions which bring about this chronic, progressive type of infection have not been determined. Another type of coccidiosis is one in which the organism is maintained at only slightly varying levels indefinitely, in some cases throughout the life of the host, but without obviously harmful effects. The differences in these types of infection reside primarily in the maintenance of the coccidium population within the host. In case the tendency to gametic differentiation is pronounced, the decrease in asexual forms results in a diminishing parasite population and hence a self-limited infection. If on the other hand, there is even a slight increase in asexual forms in successive generations, the infection becomes progressive. In infection in which the organisms are maintained at more nearly constant levels for indefinite periods, it is necessary to assume a very delicate balance in regard to reproduction.

Underlying the differences in the pathogenic effects of various species of coccidia are first, the qualitative factors illustrated in the various types of injury produced, and second, the quantitative factors residing in the ability of the parasite to continue its increase. Profound injury may result in severe, acute disease, while continued multiplication furnishes an even more serious, chronic and progressive disease.

In the absence of humoral reactions, something may be learned of immunity through the study of tissue-reactions of immunized hosts to infective forms in stained sections. For example, in immunized chickens, the sporozoites invade the epithelial cells, but the failure of the latter to respond favourably results in the prompt degeneration and disappearance of these forms. It is possible, although not demonstrated, that development may be arrested at other stages. That a great proportion of the sporozoites may be lost in consequence of lodging in cells other than those of the epithelium, is of interest. Thus the "actual" dosage, i.e., the number of infective forms reaching their normal destination may by no means conform with the "intended" dosage, i.e., the number inoculated.

Prof. E. Reichenow (Hamburg): *Ueber Coccidieninfektionen bei Mensch und Kaninchen.*

(1) Wie von Wenyon auf Grund des Studiums der älteren Literatur angenommen worden war und vom Vortragenden durch

einen Stuhlbefund bestätigt wurde, kommen beim Menschen zwei durch Entwicklungsweise und Morphologie unterschiedene Arten der Gattung *Isospora* vor. Die grosse Seltenheit der Coccidieninfektionen des Menschen legt den Gedanken nahe, dass diese Parasiten ausser dem Menschen vielleicht noch einen anderen Wirt haben. Die von Wenyon *I. belli* benannte Art ist jedoch von allen anderen bisher bekannten Arten der Gattung, insbesondere denen unserer Haustiere, deutlich verschieden; auch waren Uebertragungsversuche auf junge Katzen und Hunde erfolglos. Es wäre noch zu versuchen, ob etwa Affen empfänglich sind. Für die zweite Art, *I. hominis* (Railliet et Lucet), hat der Vortragende nachgewiesen, dass die reife Oocyste morphologisch mit der von *I. bigemina* aus dem Hunde übereinstimmt. Material für Infektionsversuche stand bei *I. hominis* bisher nicht zur Verfügung. Die Frage, ob *I. bigemina* für den Menschen infektiös ist, könnte durch Infektionsversuche mit dieser Art bei Affen der Lösung nähergebracht werden.

(2) Die durch die Infektion der Kaninchenleber mit *Eimeria stiedæ* hervorgerufenen umfangreichen Wucherungen beanspruchen infolge ihrer Eigenart ein besonderes pathologisch-anatomisches Interesse. Ihre Entstehung, die bisher nicht zutreffend beschrieben worden ist, hat der Vortragende in seinem Laboratorium eingehend untersuchen lassen (Dr. W. Minning). Die erste Reaktion, etwa drei Tage nach experimenteller Infektion, besteht in herdförmigen Lymphocyten-Infiltrationen in der Umgebung der Gallengänge. Diese treten auf, bevor Entwicklungsstadien der Coccidien in den Epithelzellen nachweisbar sind; sie sind also wohl durch den Reiz bedingt, den die auf dem Wege aus dem Blutgefäss in die Gallengangwandung befindlichen Sporozoiten im Gewebe ausüben. Diese Infiltrationen bilden offenbar ein günstiges Medium für eine starke Vermehrung der Epithelzellen der Gallengänge an den betreffenden Stellen, denn die Zellteilungen setzen schon beim ersten schwachen Coccidienbefall des Epithels in einem Grade ein, der anfangs in keinem Verhältnis zu dem Raumbedürfnis der Parasiten steht. Die Zellvermehrung führt zu Faltungen des Gallengangepithels und zu Blindsackbildungen, die der Ausbreitung der Infektion weit vorausgehen und so gewissermassen vorsorglich den Lebensraum für die Parasiten schaffen. Auf diese Weise entsteht die ganze Wucherung aus den vorhandenen Gallengangabschnitten; Neubildung von Gallengangepithel aus Granulationsgewebe, wie von Felsenthal und Stamm angenommen, findet nicht statt. Die Vorgänge waren durch Lichtbilder erläutert.

Prof. E. R. Becker (Ames, Iowa): *Diet and the Intensity of Coccidian Infection.*

It is reasonably certain that until recently, the only known means for experimentally modifying the quantitative character of a coccidian infection were (1) the immunizing effects of previous

infection, and (2) the size of the infective dose. It is true that a number of claims have been made for particular chemicals and foods, and for various treatments to which the oöcysts have been submitted, but none of them is established on experiments carried out under entirely unobjectionable conditions. The challenge to locate the vulnerable aspects of a coccidium's existence was especially enticing, for immunological adventures in coccidiosis had also been notably discouraging. In addition, my colleague, Mr. Neal Morehouse, and I had shown to our satisfaction, by means of oöcyst counts, that in at least one infection, viz., *Eimeria miyairii* in the rat host, general vitamin and mineral deficiency, vitamin-A deficiency, vitamin-D deficiency, high-casein diet, high-starch diet, skim-milk supplements to the ration, iodine supplements, vinegar supplements, high-salt content of the ration, low salt content—all these dietary modifications left the numerical increase of the parasite in its host unaffected.

Continuing our studies, it was determined that a rat which had been on a ration deficient in both vitamins B and G for 9 or 10 days previous to the date of experimental infection, would discharge but a fourth or fifth as many oöcysts as one on a diet adequate in respect to these vitamins and receiving the same amount of infective material. The immunizing effects of small doses of the parasite seemed to be as strong in the one host as in the other. This experiment was, I believe, the first clearly proved demonstration of a controlled effect upon the reproduction of a coccidium by any manipulation other than those concerned with previous infection and size of infective dose. It was rather disappointing, to be sure, that the restriction of the parasite's development was brought about by a dietary deficiency rather than by an invigorating supplement.

Later it was shown that most of the observed effect was due to the absence of vitamin G from the ration, and that vitamin B was probably not very much concerned. It was plain during this preliminary work that the failure of the parasite to develop normally was due to the absence of some stimulant from the ration, for the addition of powdered yeast, either autoclaved or unautoclaved, provided conditions favourable for more abundant reproduction. Naturally, the young host on a diet deficient in such an important factor as vitamin G does not develop normally, and it suffers inordinately from its reduced coccidian infection. In the hope that there might be located sources of vitamin G which would provide fairly good host growth, and at the same time lack the coccidium stimulant, attention was turned to liver and skim-milk. In this choice of materials we were exceeding fortunate, particularly with the liver.

Among a number of experiments in which powdered pork liver was utilized as the source of vitamin G, the following was one of the most impressive. Nine rats were given the basal ration supplemented with 10 parts of dried and powdered pork liver, and 9 the

basal ration supplemented with 10 parts Fleischman's powdered yeast. During the first 22 days on the diets, each group made a mean gain of 72 gm. The rats on the liver passed about 40 million oöcysts each; the controls, 138 million, or about 3½ times as many.

A number of experiments with chicks experimentally infected with *Eimeria tenella* have given even more striking results. Those receiving liver as the sole source of vitamin G, passed fewer oöcysts, and suffered less hæmorrhage and mortality. Oöcyst counts in chickens, however, are not so satisfactory as in rats, on account of the tendency for the cæca to plug, and thus prevent the appearance of the oöcysts in the droppings until they have somewhat disintegrated.

So far as powdered skim-milk is concerned, we have found that when it is used in rats as the sole source of vitamins B and G in the ration in the proportion of 7 per cent., there is also a marked reduction in the numbers of oöcysts passed, but the growth of the host is poor. Skim-milk, however, will receive more attention from us in the future.

How are these results to be explained? Time does not permit me to review all the evidence for the statement, but we believe that certain materials contain more of a thermo-stable coccidium-growth-stimulant than others. Yeast and grain mixtures contain very much. Liver and skim-milk contain very little. Wheat germ is rich in this substance, but not so rich as yeast; while rice polishings contains considerably more of it than liver or skim-milk, but not so much as wheat germ. When yeast or wheat germ are added to rations low in coccidium-growth-promoting properties, the multiplication of the parasite is accelerated. It seems, then, a logical tentative assumption that low parasite growth on certain diets is due to the lack of a substance that the parasite requires; although, when liver is used as the source of vitamin G, the host may not suffer visibly from a deficiency, at least during the period required to demonstrate the effect on the coccidium. We are calling this hypothetical coccidium-growth stimulant *coccidibios*.

Prof. G. Lavier and **Dr. Ch. Bondois** (Lille): *La Coccidiose du pigeon-voyageur dans le nord de la France.*

La coccidiose intestinale est très fréquente dans les colombiers du nord de la France. La recherche systématique des oöcystes dans les déjections nous a permis d'en rencontrer dans plus de la moitié des examens. Il y a d'ailleurs une légère variation de fréquence suivant la saison, variation qui est due moins à l'intervention de facteurs climatiques qu'au cycle même d'activité du pigeon-voyageur. Voici les pourcentages que nous avons relevé au cours de l'examen de 690 animaux: de novembre à janvier (période de repos du pigeon): 51 pour cent; février-avril (période d'élevage): 57 pour cent; mai-août (période des concours): 53 pour cent; septembre-octobre (période de vagabondage "aux

champs ") : 61 pour cent. Si, comme celà se pratique souvent dans certaines circonstances, on enferme les pigeons en volières, la promiscuité fait monter considérablement ces chiffres et nous avons noté alors 94 pour cent d'examens positifs.

Les infections intenses sont de beaucoup les plus rares (de 2 à 6 pour cent seulement du total des animaux), et il est évident que dans la grande majorité des cas le parasitisme est parfaitement bien toléré et reste compatible avec un excellent état de santé et de brillantes performances sportives.

L'infestation est très précoce ; elle se fait chez le pigeonneau pendant allaitement. Nous avons suivi de près à ce sujet 10 pigeonneaux tous issus de couples différents : l'apparition des oöcystes dans leurs déjections se fit pour 1, au 6ᵉ jour ; pour 3, au 8ᵉ jour ; pour 2, au 10ᵉ jour ; pour 3, au 12ᵉ jour ; pour le dernier enfin, au 15ᵉ jour. Tous ces pigeonneaux, par la suite se développèrent normalement tout en continuant à présenter des oöcystes en faible quantité.

Deux d'entre eux furent utilisés pour des expériences de surinfestation par des cultures contenant des oöcystes segmentés ; cette expérience fut faite à l'âge d'un mois pour l'un, de trois mois pour l'autre. Tous deux réagirent de la même façon : diarrhée, amaigrissement pendant 8 à 10 jours, puis retour des selles à la normale et convalescence avec relèvement rapide de la courbe de poids ; ces animaux, sacrifiés par la suite, ne montrèrent que quelques granulations très discrètes de la muqueuse intestinale. La primo-infection naturelle des pigeonneaux les prémunise donc contre une surinfection massive.

La forme sphéroïde des oöcystes, leurs dimensions (10 à 17μ sur 12 à 25μ), la localisation des formes intra-épithéliales au début de l'intestin grêle montrent qu'il s'agit, comme chez le pigeon domestique d'*Eimeria pfeifferi*.

La vitesse de segmentation des oöcystes est fonction de la température : à 0°, elle ne se produit pas, mais même après 10 jours à cette température, les oöcystes sont encore vivants, car reportés à 20°, ils sont encore capable de se segmenter ; à 15°, le cytoplasme présente après 12 heures des modifications nettes, au 3ᵉ jour, les spores sont individualisées, les sporozoïtes ne sont entièrement formés qu'au 7ᵉ ou 8ᵉ jour ; à 20° la cadence est sensiblement la même ; les sporozoïtes sont mûrs au 7ᵉ jour ; à 25°, température qui parait être optima, les spores sont formées au 3ᵉ jour et les sporozoïtes achevés au 6ᵉ ; à 38°, il se produit dans les 48 premières heures une modification du cytoplasme qui fait croire que la segmentation s'amorce, mais tout en reste là et si, après 10 jours, on reporte la culture à 20°, rien ne se produit. La température de 38° est donc léthale.

Il n'existe pas de thérapeutique spécifique pour cette coccidiose non plus d'ailleurs que pour les autres. Mais la prophylaxie est possible. L'influence des seuls soins d'hygiène est attestée par la

bénignité même des infections dans les colombiers bien tenus. La dessiccation, l'insolation ont sur les oöcystes émis dans le milieu extérieur de puissants effets destructeurs.

Nous avons expérimenté l'action léthale sur les oöcystes de divers agents physico-chimiques ; parmi ceux-ci un s'est révélé fort intéressant à la fois par son activité, sa non-toxicité pour les pigeons et son faible prix, c'est le simple sel marin : des solutions de chlorure de sodium à 15 pour cent ou plus, tuent rapidement l'oöcyste par déshydratation ; dans le liquide même le résultat est obtenu après un contact d'une vingtaine d'heures ; mais si l'on procède par arrosage, le processus est beaucoup plus rapide, surtout si l'on fait agir en même temps la chaleur et l'aération qui donnent ainsi des concentrations rapidement croissantes en contact avec les oöcystes ; ceux-ci sont ainsi tués en 15 minutes.

Une prophylaxie, toutefois, si bien conduite soit-elle, ne peut avoir la prétention de faire disparaître entièrement l'infection coccidienne du colombier Un tel but n'est d'ailleurs pas désirable, car le pigeon ne manque pas d'occasions de s'infester au dehors. Mais une bonne hygiène en diminuant la masse virulente, assure au pigeonneau dès ses premiers jours la maladie discrète qui le prémunisera par la suite contre les réinfestations massives.

Prof. E. Reichenow (Hamburg).

Der Einwand, dass es sich bei meinem Cystenbefund im menschlichen Stuhl um zufällige Passage der Cyste eines Hundecoccids gehandelt haben könnte, wird durch die Struktur der Cyste widerlegt. Bei den *Isospora*-Arten vom *Bigemina*-typus umgibt die Cystenhülle die beiden Sporen als ein äusserst zartes Häutchen, das sehr leicht zerreisst, sodass man die Sporen grösstenteils schon einzeln im Hundekot findet. In meinem Falle hingen die Sporen noch zusammen, und es ist nicht vorstellbar, dass eine reife Cyste aus dem Hundekot noch den Darm des Menschen passiert hätte, ohne dass die zarte Cystenhülle zerstört worden wäre.

Prof. J. Rodhain (Antwerp) also took part in the Discussion.

SECTION 6

SEROLOGY AND IMMUNOCHEMISTRY

MISCELLANEOUS PAPERS

Chairman : Dr. P. Hartley (London).

Prof. A. Pettersson (Lund) : *Die Beziehung des Betalysins zu den davon beeinflussten Mikroben.*

Von dem Immunbakteriolysin wird der Immunkörper durch homologe Mikroben bei 0° Grad C adsorbiert und kann mit diesen aus dem Serum entfernt werden, während das Komplement zurückbleibt. Das Normalserum verhält sich in dieser Hinsicht in ähnlicher Weise. Mackie und Finkelstein behaupten, dass die Gram-beständigen Mikroben eine der Adsorption des natürlichen bakteriziden Immunkörpers ähnliche Reaktion aufweisen, indem sie bei 0° Grad C das Bakteriolysin adsorbieren, das dann bei höherer Temperatur seine deletäre Wirkung auf die als Adsorbens benutzten Mikroben entfalten kann. Das Verhalten des Betalysins zu den dagegen empfindlichen Mikroben wurde von mir näher untersucht. Es stellte sich heraus, dass sie beim Verweilen im Serum bei 0° Grad C. in kurzer zeit getötet wurden. Der Einfluss der Temperaturerniedrigung auf die bakterizide Wirkung des Serums war gering, in einzelnen Fällen wahrscheinlich ein wenig verlangsamt. Auch mit weit grösseren Mengen Bakterien als die, welche von dem zur Adsorption genommenen Serum bei +37° Grad C. vernichtet wurden, fand bei 0° Grad C. keine grössere Adsorption des Betalysins statt ; das von den Mikroben befreite Serum wirkte fortwährend bakterizid. Dies zwingt zu der Annahme, dass das Betalysin nach dem Einwirken auf die Mikroben wieder frei wird.

Das Serum erlitt jedoch eine Veränderung durch die Adsorption. Das Serum wurde dadurch soviel aktivierender Substanz beraubt, dass die vorher zur Reaktivierung hinreichende Menge Serum nicht mehr dazu genügte. Die Aktivierbarkeit des Serums war weit weniger reduziert. Man müsste folglich annehmen, dass die sogenannte aktivierende Substanz grössere Affinität zu dem Mikroben zeigt als die, welche man aktivierbare benannt hat.

Früher war man im allgemeinen geneigt anzunehmen, dass die sogenannte aktivierende Substanz den aktiven Teil des Betalysins ausmachte und verglich sie mit dem Komplement. Wenn man noch immer einen Vergleich mit dem Alexin oder dem Immunbakteriolysin anstellen will, so müsste die aktivierende Substanz eher dem bakteriolytischen Immunkörper entsprechen.

Prof. S. Metalnikov (Paris) spoke on the Rôle of the Nervous System in Immunity and Anaphylaxis.

Prof. F. Maignon (Paris): *Recherches sur le mécanisme de la sensibilisation anaphylactique. Rôle des ferments dans l'anaphylaxie.*

Les protéines étrangères introduites dans le sang doivent être dégradées par un mécanisme analogue à celui qui est réalisé dans les cavités digestives, c'est-à-dire que la digestion se ferait en deux temps, sous l'action successive de deux ferments distincts ou de deux catégories de ferments, le premier aboutissant au stade peptone et ne le dépassant pas, et les seconds poussant la dégradation jusqu'au stade amino-acide.

Nous pensons que, dans l'anaphylaxie, les produits toxiques correspondent aux premiers stades de l'hydrolyse, ou à leurs dérivés. Ces stades toxiques seraient le résultat de l'activité du premier ferment, qu'il s'agisse d'un ferment apparu de toute pièce ou de l'activation de l'alexine par apparition d'une sensibilisatrice, d'un anticorps, d'un ferment activant, qui jouerait alors un rôle analogue à celui de l'entérokinase vis-à-vis de la trypsine. Ce premier ferment, qu'il s'agisse d'un ferment digestif ou d'une kinase, serait en quelque sorte le ferment producteur de poison. Les seconds ferments achèveraient la dégradation jusqu'aux stades atoxiques, et seraient destructeurs de poison.

Une première injection ferait apparaître ces deux catégories de ferments. L'état de sensibilisation serait dû à la persistance du premier ferment, les seconds disparaissant les premiers en raison de leur spécificité beaucoup moins étroite qui leur permettrait d'être utilisés à la destruction des polypeptides d'usure.

Nous avons pu, en effet, extraire du sang de chiens sensibilisés au sérum ou à l'ovalbumine, une diastase qui injectée à des sujets neufs (chiens, cobayes) transmet immédiatement l'anaphylaxie passive.

Du sang de chien en état de présensibilisation anaphylactique, six jours après l'injection sensibilisante, nous avons pu extraire le mélange de deux ferments. En adsorbant le premier par de l'antigène coagulé, nous avons isolé le second dans le filtrat, dont l'injection à des sujets sensibilisés a toujours produit la diminution des manifestations anaphylactiques par rapport aux témoins, voire même leur suppression.

Prof. J. Leont'ev (Moscow) [Communicated by Dr. E. W. Todd (Belmont, England)]: *Studies on Non-shock Proteins.*

(1) By means of adequate methods, special protein preparations—protoacids—are obtainable from various biological objects, such as milk, seeds of plants, etc., and the behaviour of these protoacids is just like that of the genuine proteins.

(2) Protoacids, contrary to protein preparations obtained by usual means from the same source, provoke no anaphylactic shock with guinea-pigs.

(3) Protoacids applied to other animals, such as dogs, cats, pigeons, frogs, etc., give no anaphylactic reactions either.

(4) The possibility of obtaining non-shock proteins opens a wide field for investigation, both theoretical and practical.

***Dr. V. A. Lubarsky** (Moscow) : *Sur le mécanisme de l'allergie infectieuse.*

(1) Les expériences sur les cobayes confirment les données d'après lesquelles l'hypersensibilité tuberculinique atteint, au cours de la maladie, son plus haut degré au bout de 4-5-6 semaines, après quoi elle commence à fléchir graduellement. Le maximum de l'allergie ne coïncide pas avec celui de l'immunité.

(2) Dans un certain nombre de cas les expériences ont permis de faire passer aux cobayes sains l'hypersensibilité tuberculinique des cobayes tuberculeux. Pour obtenir ce résultat on a eu recours à une injection intracutanée aux cobayes sains du sérum d'un cobaye tuberculeux. Au bout de 24 heures à la même place a été pratiquée une injection tuberculinique.

(3) La réinfection intraveineuse des souris blanches antérieurement inoculées par les bacilles tuberculeux amène dans un certain nombre de cas la mort des animaux qu'accompagnent l'hyperémie et l'oedème des poumons, l'hyperémie du foie et de la rate ainsi que des nécroses isolées dans le foie.

(4) C'est surtout par la voie rénale que sont éliminés les bacilles tuberculeux de la réinfection. En même temps on peut constater l'accélération de la destruction des bacilles de la réinfection.

Prof. T. V. Simitch and **Dr. V. Schwarz** (Belgrade) : *Effet bactéricide du sérum des tuberculeux sur les bacilles de tuberculose.*

En étudiant l'effet bactéricide du sérum *in vitro* des personnes saines et malades de tuberculose, les auteurs sont arrivés aux résultats suivants :

1. L'effet du sérum provenant de personnes saines, au cours d'une heure, est minime. Il ne s'est manifesté que dans un nombre minime de cas, sous forme d'un ralentissement insignifiant de la croissance des bactéries traitées au sérum.
2. Un effet beaucoup plus fort a été observé chez les sérums de tuberculeux. Presque dans tous les cas, on a obtenu un effet bactéricide. Certains phénomènes intéressants ont été remarqués à cette occasion. Tandis que l'effet bactéricide obtenu avec des sérums de malades, et en particulier de malades afébriles se montrait comme très faible — *l'effet des sérums provenant de malades constamment fébriles était, dans tous les cas (à l'exception d'un seul) manifestement fort et en même temps tellement permanent*

qu'on pouvait pour ainsi dire prévoir à l'avance le résultat que donneraient de tels sérums.

3. L'effet bactéricide des sérums, se manifeste encore plus fortement en utilisant certaines substances chimiques, telles que l'antiformine (10 pour cent), H_2SO_4 (10 pour cent), et NaOH (4 pour cent), par conséquent, de produits chimiques qu'on utilise aussi pour l'isolement des bacilles de tuberculose du sputum, lesquels n'ont eux-mêmes aucun effet, même après 2 heures. A cette occasion, cet effet s'est manifesté dès la 15éme minute d'expérience.

En étudiant d'une manière plus détaillée la nature de cet effet, les auteurs ont constaté qu'il était assez labile, car le sérum le perdait aussi bien en cas de stagnation prolongée qu'en cas d'échauffement à 56° C. En outre, il ne se manifeste que dans le sérum de l'homme tuberculeux. Les sérums de cobayes et lapins contaminés par des bacilles de tuberculose, même des animaux (chèvres et chiens) naturellement résistants contre la contagion, se sont montrés complètement inactifs.

En traitant les bacilles avec du sérum combiné avec des produits chimiques, il semble qu'on arrive à une totalisation de l'effet, car on obtient les mêmes résultats aussi bien en traitant les bacilles par le mélange du sérum et des produits chimiques, qu'en les traitant successivement par le sérum et l'agent chimique ou *vice versa*.

Dr. Annis E. Thomson and **Miss Agnes C. Hamann** (New York) : *The Gonococcus Complement-fixation Test : Further Study on Complement Titration.*

In a recent paper[1] the necessity was shown of applying a titration giving the complement characteristic of fixation by an immune serum-antigen complex as well as its hæmolytic power. The desirability of using a two plus control rather than a four plus control was indicated.

Instead of beginning the titration with 0·05 c.c. of 1 : 15 diluted guinea-pig complement in the combined system titration, we now include 0·01 c.c. to 0·04 c.c., for if complete fixation of these small amounts does not occur the immune serum-antigen reaction must be lacking.

If the dilution of complement is gauged by the hæmolytic system only, and two units or more of complement are used for the diagnostic test, very often the weakly positive readings are lost, and a serologically positive diagnosis is not obtained as early in the disease as when the complement is correctly adjusted. Especially when the hæmolytic titration of complement indicates the use of a solution below 1 : 15 the apparently minute amount of reacting substance present in weakly positive sera will escape detection.

[1] *J. Immunol.*, 1935, 29, 249.

This gives negative readings where a plus-minus or a one plus is of importance when correlated with the bacteriological and clinical findings.

Menck[2] advises the use of one unit of complement according to the Schuetz-Schubert method. We encountered difficulties when only one unit was added in the test proper if the complement was used in a high dilution. Price[3] improved the test by introducing a new antigen and modified technique.

In recent tests on sera, two methods of titrating the complement were re-compared. The dilution of the complement to be used in the test proper was estimated by the hæmolytic titration and by the standardized combined-system titration. In these tests a number of known negative sera were always included; the antigen control showed no anti-complementary reaction in four times the amount used in the test proper, i.e., diagnostic test. This demonstrated the greater sensitiveness of the combined system titration.

We believe that greater uniformity in results could be secured if frozen and dried standarized complement were available. Flosdorf and Mudd[4] have been successful with their method which improves on that of Cragie[5].

If complement (individual or pooled) is frozen and thawed slowly without shaking, a separation into several merging layers occurs. Small bottles containing complement frozen solidly were taken from the freezing unit, and left at room temperature until the complement had thawed. The almost colourless top layers were carefully drawn off and the light red middle layers as well as the dark red bottom layers were separated by the means of a pipette. These layers differed in strength, the top layer giving the lowest titre and the bottom layer the highest.

By freezing, thawing, and discarding the two top layers of a weak complement a much stronger complement is secured. We find that Plant[6] and Hepple[7] have already made similar observations.

Dr. A. C. McGuinness, Dr. J. Stokes, Jr., Dr. E. W. Flosdorf and **Dr. Stuart Mudd** (Philadelphia, U.S.A.): *The Clinical Use of Lyophile Human Serum.*

Apparatus and procedure for preservation of materials by desiccation *in vacuo* from the frozen state have been fully described. This procedure is finding a surprising number of uses. Viruses, bacteria, complement, enzymes, proteins, and animal and human sera have been preserved to advantage in this form.

[2] *Kliniische Wschr.*, 1931, 10, 1359.
[3] L.C.C., *The Complement Fixation Test for Gonorrhœa*, 1935.
[4] *J. Immunol.*, 1935, 29, 389.
[5] *Brit. J. Exp. Path.*, 1931, 12, 75.
[6] *Brit. Med. J.*, 1933, i, 414.
[7] *J. R. Army Med. Cps.*, 1934, 62, 291.

Convalescent and pooled normal adult human sera preserved by the lyophile process have been employed in Philadelphia during the past two years in the prophylaxis of measles and mumps, and in the prophylaxis and treatment of scarlet fever. The results obtained have in every way been comparable to those reported by investigators who have employed sera preserved in liquid form.

The advantages of serum preserved by the lyophile process over serum kept in the original liquid form are (1) that it may presumably be kept over a much longer period of time without loss of antibodies; (2) that it may be administered in concentrated form, thus markedly lessening the degree of trauma to the tissues when large amounts are injected; and (3) that the pooling of sera from donors available at different times and places is facilitated by this procedure.

Prof. H. Sachs (Heidelberg).

Zu den Ausführungen von Fräulein Hamann möchte ich bemerken, dass es mir nicht zweifelhaft ist, dass man in der Tat die Schärfe der Komplementbindung bei Gonorrhoe durch Komplementtitration und Verwendung minimaler Komplementmengen steigern kann. Ich glaube aber, dass die Steigerung der Empfindlichkeit auch durch geeignete Beschaffenheit des Antigensubstrates erzielt werden kann. So ist es uns möglich gewesen, eine wesentliche Verstärkung der Empfindlichkeit des Witebsky'schen, von den Behringwerken in den Verkehr gebrachten Gonokokkenantigens durch Aufnahme des Rückstandes in einer verdünnten Lösung von Phenol-Glycerin zu erzielen. Phenol bewirkt im allgemeinen eine Verstärkung der Komplementbindungsreaktion. Gerade bei dem Gonokokkenantigen von Witebsky scheint der Zusatz von Bedeutung zu sein, da er augenscheinlich die Wirkung autolytischer Vorgänge paralysiert. Man kann jedenfalls derart unter Verwendung der gleichen Komplement- und Serummenge eine sehr scharfe Empfindlichkeit erreichen. Es wird natürlich Gegenstand weiterer Untersuchungen sein müssen, festzustellen, ob durch eine Kombination dieses Verfahrens mit der Komplementtitration sich weitere Möglichkeiten der Verschärfung ergeben.

Prof. E. E. Ecker (Cleveland, U.S.A.), also contributed to the Discussion.

Prof. M. Heidelberger (New York), said that he had incubated antigen-antibody precipitates for two hours at 37° both with inactivated sera and in the presence of complement, but that the amount of specific precipitate was the same in the two cases. He therefore asked whether Prof. Maignon had any direct experimental evidence that his extract actually contained proteolytic enzymes.

Prof. F. Maignon (Paris).

Réponse au Prof. Ecker.—Je n'ai pas fait d'expérience d'anaphylaxie sur des organes isolés. Dans l'anaphylaxie il y a apparition de floculats puisqu'une injection de protéine fait apparaître des précipitines, mais je ne crois pas que ces floculats soient, par leur action mécanique, la cause des accidents anaphylactiques. (1) L'injection de sérum floculé ne produit pas de crise anaphylactique. (2) L'injection d'émulsion de sulfate de baryte de A. Lumière que j'étudie en ce moment avec un de mes élèves produit des manifestations qui paraissent relever d'un mécanisme tout différent de celui de l'anaphylaxie. Le cobaye, qui est beaucoup plus sensible que le chien à l'anaphylaxie protéique, l'est moins aux injections de sulfate de baryte.

Réponse au Prof. Heidelberger.—Dans des expériences en cours nous étudions, mes collaborateurs et moi, la digestion *in vitro* d'antigène additionné du mélange alexine plus premier ferment et, sans pouvoir donner encore de réponse définitive, nous pouvons dire que les premières expériences laissent espérer un résultat positif.

DISCUSSION *on* IMMUNITY REACTIONS IN RELATION TO ANTIGENIC STRUCTURE AND VARIATION IN BACTERIA

Chairman : Prof. H. R. Dean (Cambridge).

Prof. W. W. C. Topley (London), **Opener** : *Immunity Reactions in Relation to Antigenic Structure.*

There has, within recent years, been a significant change in the approach to immunological problems. The earlier workers tended to think in terms of diseases, or of bacteria and their toxins, the term " toxins " often being used in a wide, and not very clearly defined sense. To-day, we tend to think in terms of separate antigens, and of antigenic structure. When faced with the problem of host-resistance to any bacterial infection, we attempt to analyse the total mechanism into a series of effects, some antibacterial and some antitoxic, each of which is conditioned by a specific antigen-antibody reaction. There can be little doubt that, as our knowledge extends, and particularly as we learn to replace our crude immunological reagents with purified chemical products, we shall attain a corresponding increase in our control over infective disease.

In regard to this latter point—the possibility of isolating from bacterial cells chemically purified products that will induce an effective immunity—significant results have been obtained within the last few years. Independent studies by Boivin, Mesrobeanu,

and their colleagues in Bucharest, and Raistrick, Topley and their colleagues in London, have shown that it is possible to isolate from *Bact. aertrycke*, and from allied organisms of the typhoid-paratyphoid group, immunologically active substances that have very interesting properties.

The following summary of the immunological properties of the active components that have been isolated and studied by the London group of workers includes several observations that have not yet been published in detail. The methods employed in the isolation of these fractions, and our present knowledge of their chemical structure, will be described by Dr. Stacey, Dr. Challinor and Professor Raistrick in the next communication. It will suffice to note here that the fraction referred to as F 68 is a complex substance containing a polysaccharide component, linked to another non-protein component containing nitrogen, phosphorus, sulphur and combined fatty acids. This latter component, which can be separated and precipitated by treatment with dilute acid at 100° C., is referred to as F 68/P. The fraction referred to as F 68/D + L consists of the polysaccharide component of F 68, purified after the separation of F 68/P ; while F 68/D and F 68/L are the purified dextro-rotatory and lævo-rotatory polysaccharides that can be separated from F 68/D + L by fractional alcohol precipitation.

Immunologically, F 68 possesses all the more important properties of the unfractionated bacterial cell. It is highly toxic for mice and for rabbits. In rabbits, as Delafield has shown, it induces a characteristic syndrome associated with hyperglycæmia. Injected into mice it induces a significant, but incomplete, immunity to the subsequent injection of living virulent *Bact. aertrycke*. Injected into rabbits it stimulates the production of antibodies that give characteristic O agglutination with *Bact. aertrycke*, precipitation with F 68 or F 68/P, and a significant, but incomplete, passive protection in mice.

F 68/P is moderately toxic for rabbits, but very slightly toxic for mice. Injected into mice it induces little if any active immunity against living, virulent *Bact. aertrycke*. Injected into rabbits it induces the formation of precipitins for F 68 or F 68/P, but not of O agglutinins for *Bact. aertrycke*, which suggests that this component is not freely exposed at the cell surface.

F 68/D + L is entirely non-toxic for rabbits or mice ; but when injected into mice it induces an active immunity against living, virulent *Bact. aertrycke*, of approximately the same degree as that induced by F 68. When injected into rabbits it fails to stimulate the production of detectable agglutinins or precipitins ; but the serum of these immunized rabbits confers on normal mice a passive protection of the same order as that conferred by an anti-F 68 serum, which contains precipitins and agglutinins in considerable amount.

Both F 68/D and F 68/L, the separated dextro- and lævo-rotatory components of F 68/D + L, are able to induce in mice an active immunity to living, virulent *Bact. aertrycke.*

There can be no reasonable doubt that F 68 represents the O somatic antigen of *Bact. aertrycke* as it exists in nature. It has thus been shown that the main surface antigen of this organism is identical with its main endotoxin. The demonstration that the separated, non-toxic, polysaccharide component of this antigen is able to induce active immunity, and stimulate the production of protective substances, though it fails to stimulate the production of detectable agglutinins or precipititins, raises points of obvious theoretical and practical interest.

It has recently been possible to isolate from a mouse-virulent strain of *Bact. typhosum* fractions corresponding to the F 68, F 68/P and F 68/D + L fractions of *Bact. aertrycke.* Preliminary studies have shown that F 68 is toxic for mice, and induces an active immunity; while F 68/D + L is non-toxic, but induces an active immunity of the same order. It has also been found that the F 68 fraction from the mouse virulent strain shows certain chemical differences from the F 68 fraction from a smooth strain that is avirulent for mice. These differences are described by Dr. Stacey, Dr. Challinor and Professor Raistrick. Whether they are in any way connected with the presence, in the virulent strain, of the Vi antigen of Felix and Pitt, it is at present impossible to say.

It may be noted that the F 68 and F 68/D + L fractions from *Bact. aertrycke* are immunizing in extremely small doses, 10^{-6} mgm. or less. This raises a doubt as to whether the properties of the F 68/D + L fraction may not be due to traces of contamination with unaltered F 68; but, though we cannot at the moment finally exclude this possibility, it seems to us unlikely, if only because of the very different properties of the antisera produced in rabbits.

Dr. M. Stacey, Dr. S. W. Challinor and **Prof. H. Raistrick** (London): *The Isolation and Chemical Properties of an Antigen from* Bact. aertrycke, Bact. enteritidis *and* Bact. typhosum.

The various chemical fractions, the immunological properties of which have been described in the preceding abstract, were prepared as follows: A crude antigen was isolated from killed *Bact. aertrycke* bodies by tryptic digestion followed by precipitation with alcohol (68 per cent.). Purification to a chemical entity was effected by: successive precipitations from aqueous solution with alcohol (68 per cent.); removal of calcium (as oxalate); repeated precipitations from acetic acid solution with alcohol (68 per cent.); exhaustive fractionation from aqueous solution with alcohol. Pure antigen (F 68 = 6 per cent. of total bodies) was free from intact protein, gave opalescent aqueous solution, and contained N, P and organic S.

Hydrolysis of F 68 (N/10 acetic acid, 100° C.) gave a flocculent precipitate, F 68/P, and clear solution. From this solution two chemically homogeneous polysaccharides, $[\alpha]_{5461}$ + 112° and $[\alpha]_{5461}$ − 37°, were isolated. Each formed clear aqueous solutions.

The lævo-rotatory polysaccharide hydrolysed (HCl) in two stages, (1) yielding crystalline *d*-galactose, a nitrogenous carbohydrate and a "stable" acid polysaccharide, $[\alpha]_{5461}$ − 13°, which, (2) hydrolysed to *d*-galactose and an uronic acid.

The dextro-rotatory polysaccharide contained P, hydrolysed in two stages and yielded mucic acid on oxidation. The insoluble precipitate, F 68/P (N = 10·3 per cent.) was an acid, contained organic P and S and on hydrolysis (N/10 HCl) yielded reducing sugars, a fatty residue, and other products.

Analogous results were obtained with *Bact. enteritidis* and with avirulent typhoid bacillus 901.

An antigen somewhat similarly isolated from virulent typhoid bacilli behaved differently when hydrolysed with acetic acid. No flocculation of an insoluble precipitate occurred. Prolonged modified treatment yielded finally an insoluble precipitate together with a series of polysaccharide fractions $[\alpha]_{5461}$ + 14° to $[\alpha]_{5461}$ + 107°.

Prof. A. Boivin (Garches, France) : *Sur l'existence, dans certaines bactéries, d'une substance non protéique, toxique, spécifique et antigénique. Antigènes "complets" et antigènes "résiduels."*

En traitant les bactéries par un déprotéinisant, comme l'acide trichloracétique, on peut extraire de beaucoup d'entre elles, Typhique, Paratyphiques, Colibacilles, Dysentériques, Proteus, Pyocyaniques, Tumefaciens, etc., une substance, que nous appelons provisoirement antigène "complet," et dont les propriétés chimiques et biologiques sont remarquables.

L'antigène complet est soluble dans l'eau et il est absolument non dialysable ; il renferme un peu d'azote (2 pour cent environ) et il libère à l'hydrolyse acide : des sucres, des acides gras, de l'acide acétique et de l'acide phosphorique. Il est très fragile. Par chauffage en milieu faiblement acide, ou sous l'action d'une diastase bactérienne, il se clive en perdant ses acides gras (insolubles) et en laissant en solution sa fraction polysaccharidique, laquelle est indentique à l'antigène "résiduel" des auteurs.

L'antigène complet est toxique et il constitue la fraction principale de l'endotoxine des bactéries. L'autre fraction de l'endotoxine paraît être de nature protéique. Il est spécifique et il est pleinement antigénique, puisque son injection à l'animal déclanche l'apparition d'anticorps : précipitines, agglutinines et antitoxines. Il semble représenter l'antigène somatique O, responsable de la spécificité de type des bactéries. L'antigène résiduel est non toxique, il est spécifique ,mais injecté à l'animal, il ne provoque pas l'apparition d'anticorps.

L'antigène complet n'existe pas dans toutes les formes d'une

même bactérie : sa présence va généralement de pair avec l'aspect " Smooth " des colonies. Le plus souvent, l'antigène complet n'est pas accompagné par de l'antigène résiduel, dans les bactéries non autolysées. Certaines bactéries, comme les pneumocoques, qui renferment peut-être un antigène complet exceptionnellement fragile, ne livrent à notre technique que des polysaccharides spécifiques libres de toute combinaison avec des acides gras.

Prof. J. Tomcsik and **Dr. G. Ivanovics** (Budapest) : *Effect of the Anthrax Specific Substance in Infected and Passively Immunized Mice.*

The body of anthrax bacilli contains a polysaccharide specific substance, whereas the capsule consists mainly of a nitrogeneous substance, showing no reducing power after hydrolysis. This substance—denoted by us simply as P substance—gives highly specific reactions with the sera of rabbits hyperimmunized with killed encapsulated anthrax bacilli.

These sera protect mice against anthrax infection, and the immunizing power can be absorbed *in vitro* by producing a precipitate with the P substance. More than 500 mice were infected, partly with attenuated, partly with fully virulent, anthrax bacilli, and no serum gave marked protection except that containing a considerable amount of anti P immune-body. No protection was observed using the same technique with any of the commercial immune-sera obtained from many different laboratories.

Since the protecting action of our immune-serum was strictly parallel with its anti P immune-body content, it seemed worth while to study the outcome of infection in passively immunized animals, if purified P substance was injected. It was expected that some light might be thrown on the existence of "aggressin"-like substances and upon Bail's theory of the mechanism of anthrax infection.

If mice, previously protected with 1 c.c. of anti P immune-serum, were infected with 20 lethal doses of an attenuated strain A.15, then 1 mg. of the purified P substance neutralized the protecting action of 1 c.c. immune-serum. It appears, furthermore, that the sooner the P substance is given, the stronger is its neutralizing action.

The minimum neutralizing dose of P substance, given one day before the infection, was 0·05 mg. ; still considerably higher than the amount giving " optimum precipitation."

No experiments were made with anthrax œdema fluid. We are convinced, however, that its effect would be similar, since it contains a large amount of P substance. So the alleged aggressin nature of the œdema fluid, according to the interpretation of Bail, could be partly verified.

Our results seem to prove that a hapten-like substance might influence the outcome of an infection.

Dr. A. Felix (London): *The Significance of Different Bacterial Antigens in Infection and Immunity.*

It is now being more generally recognized that different antigenic components of the bacterial cell play distinct and separate rôles in the pathogenicity and immunogenic activities of bacteria. With regard to the known *in vitro* immunity reactions, it is well established that in agglutination and precipitation both the H and O antigens of flagellated bacteria produce their separate and distinct effects, whereas in complement-fixation and bactericidal and opsonic action the effect of the interaction between the H antigen and H antibody is entirely negligible. This is in harmony with the fact that this antigen and its corresponding antibody are of no importance in active or passive immunization against infection with flagellated organisms.

B. typhosum provides another illuminating example of the diversity of functions assigned to the various antigenic constituents of the bacterial cell. The pathogenicity of the typhoid bacillus is determined by, or closely associated with, the combined activity of the two antigenic components known as the O and Vi antigens. The smooth variant which contains the O but is devoid of the Vi antigen, and the rough Vi-containing variant, which is devoid of the O antigen, both represent types of low virulence. Yet the Vi antigen alone is required to induce a state of efficient immunity against experimental infection of mice with highly virulent strains which possess the two types of antigen. It is very likely that similar complex interactions between two or more antigenic components are at play in other pathogenic bacteria and probably also in filterable viruses. It is quite conceivable that some non-antigenic constituents of the bacterial cell are also concerned in pathogenicity. How such substances can be explored immunologically is as yet uncertain.

The demonstration of the Vi antigen of *B. typhosum* was made possible by the use of living bacilli for the preparation of both the immune sera and the suspensions employed as reagents in agglutination tests. Subsequently certain chemically treated substitutes could be made available. This procedure is suggested for similar studies of other bacterial species. The earlier view, still held by many bacteriologists, that suspensions of killed bacteria, prepared according to certain routine methods, are in all respects adequate substitutes for the "natural" antigens contained in the living organisms, is certainly out of date and deserves to be abandoned.

Dr. H. Habs (Heidelberg): *Bedeutung der Antigenstruktur für die Serodiagnose der Brucellosis.*

Bei der serologischen Diagnose der Brucellosis bedeutet die Beachtung der Dissoziationsvorgänge und der Antigenstruktur der

Brucellen, die vor allem durch die Arbeiten von G. S. Wilson geklärt worden ist, einen wesentlichen Fortschritt. Mehr noch als in anderen Bakteriengruppen besteht die Möglichkeit, dass Laboratoriumsstämme in die Rauhform übergehen und hierdurch unspezifische Reaktionen vortäuschen. Wenn in der Literatur Angaben über den positiven Ausfall der Agglutinationrreaktion bei Schwangerschaft, bei Tuberkulose und anderen Erkrankungen vorliegen, so lässt sich nachweisen, dass bei derartigen Fällen einwandfreie S-Formen auch einen einwandfrei negativen Ausfall der Reaktion geben, während Stämme, die einen Übergang in die R-Form zeigen, zu positiver Reaktion neigen. Es ist deshalb eine laufende Kontrolle der Laboratoriumsstämme auf Dissoziationsvorgänge erforderlich, für die als schärfste und dabei leicht durchführbare Probe die Trypaflavinreaktion empfohlen wird.

Die verschiedenen Typen der Brucellagruppe unterscheiden sich in ihrer Antigenstruktur in prinzipiell anderer Weise von einander als diejenigen der Salmonellagruppe. Alle Typen besitzen die gleichen Teilantigene, lediglich die quantitative Verteilung ist verschieden. Es ist deshalb nicht möglich, den Agglutiningehalt eines Serums durch Auswertung mit verschiedenen Antigenen zu bestimmen. Es ist vielmehr notwendig, Absättigungsversuche in quantitativer Abstufung vorzunehmen. Auf diese Weise gelingt es, die Differentialdiagnose zwischen Melitensis- und Abortusinfektion serologisch zu stellen. Diese Möglichkeit ist deshalb besonders wichtig, weil bei der Brucellosis die bakteriologische Diagnose einer Erkrankung grösseren Schwierigkeiten begegnet als bei typhösen Erkrankungen.

Mr. P. Bruce White (London): *The Specific Polysaccharide Complex and Variation of* Vibrio choleræ.

The cholera-El Tor group is sharply marked off from all other vibrios by its heatstable polysaccharide-containing antigens. As observed by the Japanese, there are two main serological sub-types both including true cholera and El Tor strains: these sub-types have a common group antigen and each a strong specific antigen.

The cholera vibrio presents three grades of specific polysaccharide structure:

(1) The S form, presenting 4 non-protein carbohydrate-containing serologically-active constituents or groups of constituents, Cα, Cβ, Cγ and Cδ. Of these, Cα determines both the agglutinative specificity of *V. choleræ*, and by internal differentiation, the differences between the Ogawa and Inaba sub-types of this organism.

The O antiserum of smooth *V. choleræ* is essentially anti-Cα.

(2) The R form, in which Cα is suppressed or lost and in which rather wider serological relationships are disclosed. Its O antiserum is essentially anti-Cβ.

(3) The ρ-form which presents more or less generalized agglutinative properties and, having lost both Cα and Cβ, possesses Cγ and Cδ only. Its O antiserum is anti-Cγ and Cδ or anti-Cδ.

R forms may be isolated from normal S cultures by exposing these to activated S antiserum (or to A-type choleraphage) ; ρ forms by exposing R cultures to activated R serum. The action of activated antiserum and choleraphage A in assisting isolation of these forms is merely to select insusceptible " loss " variants, continuously generated during normal growth of the culture. R and ρ variants, by whatever means isolated, are resistant to A-type choleraphage.

When vibrios are digested with papain, Cα and Cγ are brought into solution : Cβ and Cδ are not, but may be liberated from the residue by alkali. Cα may be separated from Cγ and differs from the other C substances in being readily destroyed by alkali.

As regards vibrios in general : the Cα series of substances are type specific ; the Cβ substances divide the types into groups, while the Cγ and Cδ substances appear to be common to all strains.

There has been an attempt to represent this type of variation (HO→O, S→R, R→ρ) as cyclic : I regard it as purely degenerative. How much further the vibrio may shed its substance and what other changes it may undergo remain to be determined. Resistance " acquired " to choleraphage (types A—K) does not appear to be associated with any *positive* antigenic modification. While it is to be *presumed* that vibrios are subject to progressive evolutionary changes, I can find no basis whatsoever for the current legend of transmutation to and from the serological cholera type.

Prof. H. Braun (Istanbul) : *Die Ernährung der Bakterien und die Bildung ihrer Antigene.*

Bei Mikroorganismen sind folgende Grade der Abhängigkeit der Antigenbildung von den Ernährungsbedingungen zu unterscheiden :

(1) die exogen weitgehend unabhängige Potenz,
(2) die exogen abhängige Potenz und
(3) die endogen bedingte Impotenz zur Bildung eines Toxins oder eines somatischen Antigens (Geissel-, Kapsel-, Körper-antigens).

Diese Potenzgrade sind entweder *Arteigenschaften* oder *individuelle* Eigentümlichkeiten einzelner Stämme.

Beispiele : Paratyphus-B-Bacillen bilden ihre Antigene in den üblichen bouillonhaltigen Nährböden und in den synthetischen Medien. Friedlaender-Bacillen bilden ihre Kapseln unter primitiven Ernährungsbedingungen, während Milzbrand-Bacillen es nur bei Serumanwesenheit tun. Manche Typhus-Stämme bilden das Vi-Antigen auch auf den gewöhnlichen Nährböden, andere verlieren es unter diesen Umständen. Manche Typhus-Bacillen und Proteus-

Bacillen bilden ihr H-Antigen auch unter den günstigsten Ernährungsbedingungen nicht.

Die Ernährung kann zur Folge haben :

(1) Eine *quantitative* Aenderung der Antigene, die
  (*a*) in völligem Verlust eines Antigens,
  (*b*) in einer gegenseitigen Verschiebung der Mengenverhältnisse der Antigene bestehen kann.

(2) Eine *qualitative* Veränderung der Antigene.

Die Ernährungsbedingungen, die zur *quantitativen* Aenderung der Antigene führen, sind folgende :

(1) ungenügende Mengen der notwendigen Nahrungsstoffe ;
(2) mühsame assimilatorische Prozesse ;
(3) Störungen der Fermentfunktionen durch die Anwesenheit bakterienfeindlicher Stoffe z.B. Karbolsäure, oder durch ungünstige Temperaturen. Wasserstoffionenkonzentrationen oder durch hohen Salzgehalt.

Beispiele : Auf nährstoffarmen oder karbolhaltigen Medien bilden Typhus-, Paratyphus-, Proteus-Bacillen ihre ektoplasmatischen Geissel-Antigene nicht. In synthetischen Nährmedien ist das relative Mengenverhältnis der ektoplasmatischen Antigene zu den endoplasmatischen bei Paratyphus-B-Bacillen ein anderes als bei Bakterien, die auf bouillonhaltigen Medien gewachsen sind. Darauf beruhen die Unterschiede bei den Immunitätsreaktionen und in der Empfindlichkeit dieser Keime physikalischen und chemischen und chemischen Schädigungen gegenüber.

Qualitative Veränderungen der Keime unter dem Einfluss der Ernährung kommen selten vor.

Die Impfstoffe müssen diejenigen Antigene enthalten, welche sich in den *infektionstüchtigen Aussenweltsformen* der Keime finden und gegebenenfalls diejenigen, welche während des Kranksheitsprozesses im Organismus *neu* in den Keimen entstehen, z.B. bei Pneumokokken, Milzbrandbacillen, Pestbacillen usw. Nach diesen Gesichtspunkten müssen die Zuchtbedingungen zur Herstellung der Impfstoffe erforscht werden.

Dr. Kurt Meyer (Berck Plage, France) : *Ueber Antikörper mit multiplen Affinitäten.*

Es kann als sicher gelten, dass komplexe Antigene im Organismus die Bildung differenter, den einzelnen Komponenten des Komplexes entsprechender Antikörper, hervorrufen können. Andererseits kann aber gegen diese Antigene auch ein Antikörper mit multiplen Affinitäten gebildet werden. In diese Kategorie von Antikörpern gehören jene Forssman-Haemolysine, die durch bestimmte Bakterien (*B. shiga*, Salmonella-Gruppe) und durch menschliche Blutkörperchen der Gruppe A beim Kaninchen hervorgerufen werden. Sie haben eine specifische Affinität nicht nur zum Forssman-Receptor, sondern auch zu dem art-oder typenspecifischen

Receptor, die, wie wir für den *B. shiga* gezeigt haben, beide in einem Molekül verbunden sind. Die genannten Haemolysine werden von dem homologen Antigen (Shiga-Bacillus, A-Blutkörperchen) so fest gebunden, dass sie beim Erhitzen auf 60° nicht oder nur in ganz geringer Menge dissociiert werden, während ihre Bindung an Hammelblutkörperchen, wo sie nur mit einer Affinität reagieren können, ebenso locker ist wie die Bindung der gewöhnlichen Agglutinine, die ebenfalls nur mit einer Affinität des komplexen Antigens in Bindung treten können. Aus der ungewöhnlichen Festigkeit der Bindung wird geschlossen, dass die doppelte Affinität jener Antikörper durch das Vorhandensein zweier differenter bindender Gruppen bedingt ist. Diese Tatsache ist von Bedeutung einmal für das Verständnis der Aviditätsunterschiede der Antikörper, sodann für die Frage, inwieweit ganz allgemein den einzelnen chemischen Gruppen des Antigens bestimmte Gruppen des Antikörpers entsprechen.

Dr. F. Kauffmann (Copenhagen): *Ueber die Bedeutung der Salmonella-Antigene für die Widal-Reaktion.*

Die serologische Diagnose der Salmonella-Erkrankungen beruht in erster Linie auf dem Nachweis von Agglutininen im Patientenserum, die gegen bestimmte Salmonella-Antigene spezifisch eingestellt sind. Die genaue Kenntnis der Antigenstruktur ist daher die erste Voraussetzung für die richtige Anstellung und Beurteilung der Widal-Reaktion. Da der Mensch in der gleichen Weise wie das Kaninchen auf die Einverleibung von Salmonella-Antigenen mit Agglutininbildung antwortet, so gilt das *Kauffmann-White-Schema* der Salmonella-Antigene auch für die Widal-Reaktion, für die folgende Hauptregeln aufgestellt werden können :

(1) *Der Nachweis der O-und H-Agglutinine erfolgt getrennt* und zwar unter Berücksichtigung des *Kauffmann-White-Schema*.

(2) Beim Nachweis der O-Agglutinine ist das in Betracht kommende O-Antigen in der *Glattform* anzuwenden und zwar am besten als Alkohol-Emulsion.

(3) Beim Nachweis der H-Agglutinine sind *sehr bewegliche* Bouillonkulturen zu verwenden, die am besten mit Formalin abgetötet werden.

(4) Bei den diphasischen Salmonella-Stämmen sind beim Nachweis der H-Agglutinine sowohl die *spezifischen* als auch die *unspezifischen Phasen* zu verwenden ; doch kann man sich beim Paratyphus B im allgemeinen mit der spezifischen Phase allein begnügen.

(5) Beim *Typhus-Widal* braucht das Vi-Agglutinin *nicht* berücksichtigt zu werden, da es keine wesentliche diagnostische Bedeutung hat—im Gegensatz zu der grossen diagnostischen Bedeutung bei der Diagnose

von Typhus-Stämmen. Wenn man aber den Typhus-Widal mit lebenden Bacillen ansetzen will, so muss man beim O-Widal die W-Formen der Typhusbacillen benutzen, welche das Vi-Antigen nicht besitzen. Durch die Anwesenheit des Vi-Antigens wird nämlich die O-Agglutinabilität der Bacillen herabgesetzt oder sogar aufgehoben.

(6) Um möglichst einheitliche und international vergleichbare Resultate zu erzielen, ist die Anwendung *abgetöter* und *standardisierter Emulsionen* zu empfehlen.

Dr. R. Th. Scholtens (de Bilt, Holland) : *Qualitative and Quantitative Receptoranalysis of* Vibrio choleræ : *Comparison of Different Schemes.*

The first description of different serological types of cholera vibrios was given by the Japanese authors Kabeshima and Nobechi.

In 1933 the writer demonstrated by absorption experiments that amongst nine strains there were two types, with antigenic structures A and AB and an intermediate type which contained the antigen A and the haptene of B.

The second antigen, B, has an influence on A, as the absorbing capacity of an AB strain for the agglutinin A of a serum made with an A strain is greatly diminished. But it appears not to be lessened for the agglutinin A of a serum made with an AB strain. Therefore, as the agglutinin A from a serum of the A type and the agglutinin A from a serum of the AB type are absorbed in different strengths by the AB type, they cannot be identical, and therefore the corresponding antigens are not identical either. This is not due to the antigen A of the A strains containing fractions which the A antigen of the AB strains do not contain, but it must be explained as follows :

Landsteiner described how chemical substitutions in an antigen may cause differences in the specificity of the antigen. The antigen formed hereby still acts, but more weakly, with an immune-serum made with the orignal antigen. A similar relation exists also between the antigen A of an A strain and the antigen A of an AB strain. In this case the change is caused because the group B is fixed to the antigen A. The difference between the antigen A of the A strains and the antigen A of the AB strains is, therefore, a difference in specificity of the entire antigen, and is not due to the antigen A of the A strains containing antigen which the AB strains do not contain.

The method which is employed for showing differences of this kind is the quantitative receptoranalysis, as such differences, which were first proved to exist in artificial antigens by Landsteiner, and which at present also appear to play a rôle in natural bacterial antigens, can be demonstrated by quantitative absorption experiments.

[In the intermediate type the change of antigen A is slight. It is possible to explain this, and the fact that the B group in these vibrios is only a haptene, as follows: The B group is not fixed to the antigen; through this its action on A is slight, but through this also this group has no "Schlepper," and consequently is only a haptene.]

The three types thus are: A, AB and (A)B haptene. The brackets point out a more or less strong change of the antigen A. The scheme was compared with schemes of other authors.

Dr. R. Lovell (London): *The Significance of Normal Agglutinins.*

The view is expressed that the possession of common antigenic factors by apparently unrelated bacteria has an important bearing upon the significance of normal agglutinins. Blood sera of normal animals may contain agglutinins for many species of bacteria. As judged by absorption tests, these agglutinins are specific, and at any rate, in the case of the Salmonella group they are specific for particular antigenic components. The presence of these agglutinins is not necessarily due to previous or latent infection with the homologous bacteria, but the stimulus for their development may be provided by other species of bacteria which possess a common antigen, maybe to a minor degree only. This view gains support from the following facts:—

(*a*) Apparently healthy cattle, pigs, sheep and horses possess agglutinins for Salmonella bacteria in their sera, and in the case of pigs and sheep it has not been found possible to isolate members of the Salmonella group from the tissues or fæces of slaughtered animals possessing these agglutinins.

(*b*) Normal horses examined in this country also possess agglutinins for *Pf. mallei*, yet there has been only one case of glanders in horses reported in Great Britain in the last ten years.

Cattle, pigs and goats which do not normally suffer from glanders also possess agglutinins for *Pf. mallei* in their sera.

(*c*) The possession of antigens common to species unrelated in the systematic sense is already known, e.g., *Strept. pneumoniæ* type II; Friedlander's bacillus type B and a variety of yeast; strains of *Past. pseudo-tuberculosis* and certain members of the Salmonella group; some strains of *Bact. coli* and certain Salmonella.

(*d*) Two strains of *Bact. coli* isolated from the fæces of pigs, the sera of which possessed agglutinins for Salmonella bacteria, stimulated the production of agglutinins for Salmonella bacteria to low titres in rabbits when injected intravenously.

Further work on the sharing of antigens between bacteria otherwise unrelated may throw light on the significance of normal agglutinins.

Prof. M. Macheboeuf and **Dr. J. Dieryck** (Paris) : *Études immunochimiques sur le bacille tuberculeux.*

Parmi les lipides du bacille tuberculeux il est un haptène de fixation de l'alexine.[1] Nous avons déjà pu le purifier et l'étudier,[2] il est extrêmement actif comme haptène fixateur, mais n'est pas antigène ; il n'est pas non plus haptène de précipitation, les haptènes de ce type ne sont pas lipidiques.

Si l'on injecte à un animal des bacilles tués, il apparait, en petite quantité, un anticorps spécifique de l'haptène lipidique ; cet haptène est donc, dans les bacilles, à l'état d'antigène complet, donc probablement uni à des protéides (complexes lipidoprotéidiques antigéniques). Nous avons cherché à extraire ces antigènes complets. Pour celà il fallait éviter toute manipulation brutale dénaturant les complexes (chaleur, oxydation, acides, alcalis, alcools) ; voici notre technique :

1. Les bacilles sont lavés pour éliminer le milieu de culture.
2. Les bacilles encore vivants sont plongés dans de l'acétone purifiée froide ; puis épuisés pendant six jours par l'acétone, à froid, dans le vide. [3]L'acétone extrait : eau, pigments, divers lipides et quelques glucides, mais aucun haptène.
3. Les bacilles sont épuisés, à froid, dans le vide, par l'éther qui enlève d'autres lipides non haptènes.

Les bacilles ainsi privés de la majeure partie des lipides conservent leurs haptènes. Si on les traitait alors par de l'alcool on arracherait l'haptène lipidique, mais nous voulions les antigènes complets, aussi avons nous évité l'alcool. Les résidus bacillaires sont agités énergiquement avec des billes en ajoutant très lentement de l'eau physiologique. L'émulsion obtenue est laissée 12 heures dans une glacière, puis centrifugée énergiquement pour éliminer les résidus bacillaires. La solution contient des protéides, de l'haptène lipidique et aussi des haptènes de précipitation. Cette solution contient bien les haptènes à l'état de complexes antigéniques, car, par injection, elle fait apparaitre des anticorps extrêmement puissants (sensibilisatrice et précipitines) dont le taux est même très supérieur à celui rencontré chez les animaux tuberculeux.

On peut obtenir les haptènes libres et non antigènes à partir de la solution aqueuse des antigènes en coagulant les protéides par de l'alcool bouillant.

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[1] *Z. f. Immun.*, 1912, 14, 359 ; 1913, 15, 245 ; *Ann. Inst. Past.*, 1923, 37, 787 : 9ème *Congress Internat. Chim.* (*Madrid*, 1934), 5, 290 ; *Ann. Inst. Past.*, 1935, 54, 71.

[2] *Ann. Inst. Past.*, 1934, 53, 591 ; 1935, 55, 433 ; 1935, 55, 547.

[3] *Bull. Soc. Chim. Biolog.*, 1934, 16, 229.

En somme, nous avons montré que l'haptène lipidique qui est par lui-même inactif comme antigène, se trouve dans les bacilles sous la forme de complexes antigéniques très actifs et solubles dans l'eau. Grâce aux solutions ainsi préparées, on peut obtenir des anticorps puissants et les lapins ainsi immunisés résistent beaucoup plus longtemps que des témoins à une infection tuberculeuse expérimentale.

Dr. Marguerite Aïtoff (Paris).

Je voudrais compléter la très intéréssante communication de M. Boivin en rapportant les faits que j'ai observé en étudiant le bac. dysentérique des nouveaux-nés.

En partant des cultures microbiennes j'ai pu isoler deux substances. (1) Une exotoxine obtenue en filtrant sur bougie une culture de 15 jours en milieu liquide. (2) Une endotoxine ou lysat obtenue en émulsionnant une culture jeune sur gélose dans de l'eau distillée, puis en laissant l'émulsion pendant quelques jours à l'étuve et en la filtrant ensuite sur bougie.

La toxine s'est montrée moins active que le lysat. Celui-ci gardait sa virulence, même lorsqu'on émulsionnait des cultures devenues avirulentes.

Nous avons pu constamment obtenir la réaction de Shwartzman avec le lysat. Cette même réaction était négative, si les injections préparantes et déchaînantes étaient faites avec la toxine.

Prof. A. Boivin (Garches, France).

1. Au sujet de la communication du Dr. Macheboeuf :—J'ai suivi avec un vif intérêt l'exposé que vient de nous faire le Dr. Macheboeuf et je voudrais lui demander quelles relations il entrevoit entre la substance caracterisée par lui comme une combinaison d'un polyalcool cyclique avec des acides gras et avec de l'acide phosphorique et les phosphatides du bacille tuberculeux extraits par Anderson, phosphatides qui livrent à l'hydrolyse de l'inositol et qui présentent des proprietés biologiques fort remarquables.

2. Au sujet des observations de Mme. Aïtoff :—Les importantes observations de Mme. Aïtoff viennent confirmer les travaux d'Olitsky et Kligler sur l'existence, dans les filtrats de b. dysentérique, d'une exotoxine et d'une endotoxine. Cette endotoxine est du type glucido-lipidique usuel, comme l'ont prouvé récemment deux de mes collaborateurs, les Drs. L. Mesrobeanu et G. Calalb. En ce qui concerne la possibilité d'obtenir le " phénomène de Shwartzman" avec l'endotoxine, les intéressantes observations de Mme. Aïtoff sont à rapprocher de celles d'un élève du Prof. Tomcsik, qui a démontré que chez les Salmonelles c'est l'antigène-endotoxine qui est responsable du " phénomène de Shwartzman."

Prof. M. Macheboeuf (Paris) : *Réponse au Prof. Boivin.*

La fraction qu'Anderson appelle phosphatide " purifié " est un mélange de nombreuses substances très dissemblables que nous

avons pu séparer par des fractionnements répétés. Parmi ces substances se trouve l'haptène lipidique lui-même dont nous parlons aujourd'hui et que nous avons pu obtenir pur. Cet haptène est constitué par un polyalcool cyclique estérifié, d'une part, par une molécule d'acide phosphorique et, d'autre part, par deux acides gras. Dans le phosphatide dit " purifié " d'Anderson se trouvent en outre diverses substances parmi lesquelles sont de vrais phosphatides azotés voisins de la lécithine et de la céphaline, mais dans la molécule desquels n'entre pas de glycérol mais un autre polyalcool. Ces phosphatides eux-mêmes sont absolument inactifs comme haptènes de fixation de l'alexine.

Les propriétés haptènes de la fraction " phosphatide purifié " d'Anderson sont dues uniquement à la présence dans cette fraction de l'haptène que nous avons isolé.

Prof. H. Sachs (Heidelberg).

Zu den interessanten Mitteilungen von Herrn. Meyer möchte ich die Frage zur Diskussion stellen, ob es sich gerade bei den durch Bakterienimmunisierung erhaltenen heterogenetischen Antikörpern um Antikörper mit multiplen Affinitäten handeln muss. Man könnte auch annehmen, dass die einzelnen Bakterienarten verschiedene Partialfunktionen des ja ungemein kompliziert zusammengesetzten heterogenetischen Antigenapparates enthalten; auch dann wäre es verständlich, dass die heterogenetischen Antikörper nur von den homolgen Bakterien gebunden werden, ohne dass eine Kupplung der heterogenetischen und der artspezifischen Antikörper bestehen müsste. Im übrigen scheinen mir aber die Vorstellungen von Herrn Meyer sehr bemerkenswert zu sein, insbesondere durch de Annahme, dass die Affinität der Antikörper-Reaktion von der mehrfachen Bindung abhängt. Diese mehrfache Bindung könnte auch gegenüber einem einheitlichen Antigen erfolgen, sodass die Avidität der Antikörperwirkung eine Funktion multipler Affinitäten sein könnte. Es ist charakteristisch, dass die Dissoziation der gebundenen Antikörper bei höherer Temperatur häufig umso stärker in Erscheinung tritt, je weniger avide bezw. je weniger stark die unmittelbare Antikörperbindung erfolgt. So könnte die Möglichkeit der Wiedergewinnbarkeit der Antikörper von der Zahl der multiplen Affinitäten abhängen.

JOINT DISCUSSION *with* SECTION 1 AND SECTION 3 *on* VARIATION. RELATION OF CHANGES IN MORPHOLOGICAL AND CULTURAL CHARACTERS TO CHANGES IN CHEMICAL COMPOSITION AND TO ALTERATIONS IN ANTIGENIC STRUCTURE, TOXIN PRODUCTION AND PATHOGENICITY

Chairman: Prof. H. Zinsser (Boston, U.S.A.).

Dr. J. A. Arkwright (London), **Opener**: *Variation of Bacteria.*

Variability or the liability to change is a general character or

function of things in this world. As Shelley says "Naught may endure but mutability." Lifeless things are continually being altered by outside forces and resulting chains of change are set up. It is remarkable that living things should ever have been regarded as unchanging, as in the doctrine of the fixity of species. The idea arose from their extraordinary power of adjustment by which they apparently overcome the results of contact with the outside world. This characteristic is usually called adaptation and regarded as change in the face of an altered environment. Really the chief characteristic of living things in this respect is that they retain or recover so much of their form and complex functions, although their internal structure is greatly altered, or *vice versâ* they may change externally but remain the same in other essentials. The hereditary variability of bacteria is a well-established fact, but this has not been the accepted view for many years.

Repeated changes in biological thought during the last forty or fifty years regarding the higher plants and animals, have greatly influenced the opinions of bacteriologists in this respect. In the last century, and again more recently, the theory of Lamarck has had a wide influence, and hereditary variation has often been attributed to the direct effect of the environment. Though it can often be shown that the effects of injury are not inherited by the offspring, nevertheless, the transmission of some of the effects of the environment cannot be disproved, and an alternative explanation of their apparent inheritance is hard to find.

Darwin attributed many hereditary variations to the direct action of external conditions and to their indirect action through the sexual organs, and he believed that Natural Selection acted on such variations.

On the other hand, Weismann laid great stress on evidence which appeared to preclude any effect of external conditions on inheritance, since he regarded the germ-plasm as continuous and completely protected by the soma or body from the outside world. His views were supported by Mendel's experiments, which showed that segregated characters were inherited apparently unchanged by external influences and explained how apparent mutations might really be due to the reappearance of recessive characters. Further evidence against Darwin's views was brought forward by Johannsen who demonstrated that many small variations which had been looked on as the basis for Natural Selection were not inherited at all, but were fluctuating characters and due to chance temporary conditions. There is, however, now good evidence that many large and small variations are hereditary and that similar characters may be hereditary in one species and not in another.

Some biologists who have supported Weismann's doctrine have maintained that the germ-plasm is not only continuous but almost unchangeable, and have doubted the occurrence of mutations. These doctrines have even been applied to bacteria. It has, however,

now been shown that hereditary mutations, especially in plants and insects, are of frequent occurrence and may be induced by X rays. As recently as ten or fifteen years ago the occurrence of hereditary variations was still doubted by some bacteriologists and opposition to the acceptance of those cases which had been observed was stiffened by the excessive claims of some workers that great and irregular mutations occurred and even transmutations between races which appeared to be of the rank of species.

In order to establish the occurrence of a hereditary variant in bacteria it must be shown that the new race is really derived from the original pure stock and is not merely a contaminant or a race selected out of a culture of mixed aces. The demonstration must be made by a strict technique and the source of the variant should be supported by its general resemblance to the parent stock ; a claim of complete transformation of one race or species into another would demand especially stringent critical enquiry. In some well-observed instances the new variant has arisen in a culture too quickly and after too little multiplication for selection of a pre-existing sub-race to be a sufficient explanation of the newly-appeared variant.

Hereditary variation may concern one or more well-defined characters, e.g., the forms of the individual cells or colonies, motility, gain or loss of virulence with or without distinct change in serological characters or chemical composition. Variation of bacteria may also affect the ability to ferment certain substances or to derive nutriment from simpler or more complex culture media. Variation, either qualitative or quantitative, may occur in toxin production or in susceptibility to the action of a bacteriophage.

Many of these variations have been studied by the biochemist and appear to consist of change or adaptation of enzymes or groups of enzymes and to include cases which are very diverse. The change may involve an attack on a new substance and the formation of a new constituent of the bacterium out of ordinary culture media, or such a change may only occur when the newly-formed substance has been first added to the substrate ; but in either case, such a component of the bacterial cell, when once acquired, may be passed on by inheritance to future generations.

These different kinds of variation undoubtedly occur, and exhibit the great flexibility of the very complex biochemical processes in bacterial nutrition and growth. All forms of life are evidently very flexible, but in different degrees. The state of a family of living things is dynamic, not static, as has been insisted by B. C. J. G. Knight in the case of bacteria, and by other biologists for higher organisms.

S. C. Harland has recently given an account of the many subspecies, hybrids and varied forms of the cotton plant. He describes the continuous alterations in the genes, which change their positions in the chromosomes and also their activities in the course of hered-

itary transmission. Knight, in his recent review of the biochemical work by himself and others on bacterial nutrition, vividly describes the enzymes and other constituents of bacteria and their inheritance, showing the great flexibility of the bacterial activities. He appears to disclose more fundamental and continuous changes in the biochemical components of bacteria than are to be found in other aspects of the subject. It seems probable that the biochemical changes are really the essential processes in all variations and mutations of living things.

The birth of a new species may never have been observed, but such an observation has probably been more nearly approached in the case of bacteria than of other forms of life, on account of their great variability and of the short period of time occupied by a single generation. It might be hard to recognize a new species if it appeared, but variants of specific rank may already have been observed in the Schizomycetes.

If viruses may legitimately be included in these remarks it may be pointed out that these very minute forms show greater variability than bacteria, especially in function, just as bacteria appear to vary more than the higher forms of life.

As the body was said by Weismann to protect the germ-plasm of the higher animals from change due to the environment, so perhaps the biochemical substances essential for heredity are further protected by inclusion in the specialized genes and chromosomes, where they are less exposed than the corresponding elements in bacteria, whilst in the excessively small elementary bodies of the viruses they may on the same principles be afforded still less protection.

Dr. A. Grumbach (Zürich) : *Microbic Variability.*

Increasing importance of type diagnosis brings up again the whole question of bacterial classification, but it is as difficult to define bacterial type as bacterial species.

Despite difficulties, antigen analysis will supply the basis of order—but only with adequate collateral investigation of cultural variability.

Each microbe bears to some extent the stamp of its past. I call varieties, varietal by influence of their environment, " phases." Their recognition helps to avoid taxonomic errors, and assists understanding of the individual infectious disease.

In the individual disease species and type are less important than phase, for this determines the causation of one clinical disease by various microbes and of different diseases by one. The phase may be so marked as to be mistaken for a species (e.g., *B. bellis* Heim or *Proteus zenkeri*), or for a type, e.g., gravis, mitis and intermediate forms of *C. diphtheriæ,* which must be regarded, despite their relative stability, as phases). Since the notion of phase guards

against a misconception of type, against diagnostic errors and against confusing type and virulence, it has more than a verbal reality. Experiments with streptococci, differentiated by Warren Crowe's method, showed that virulence is often lacking in the cultural phase: discovery of the virulence antigen confirmed this observation.

To avoid error in species and type diagnosis, the slightest deviations from the norm must be considered. Recently an organism, culturally and morphologically pneumococcus-like, but inagglutinable by any of Miss Cooper's sera, was isolated: by Dr. Gundel it was considered a new type but from its peculiarities (slight mucus production and unusual vigour of growth) we suspected that its " type " was marked by its " phase ": by selecting from a series of rapidly reinoculated cultures we identified the strain with type 23.

We isolated from the fæces of a sporadic case of enteritis a bacterium in motility, staining and culture agreeing with the paratyphosus-group. It was agglutinated by certain Paratyphosus O sera (Newport and Enteritidis weakly, Paratyphosus B, Paratyphosus murium and Reading strongly) but failed to exhaust any completely; H affinities with this group were lacking. After passages and selection, it lost its Paratyphosus-like O reactions, and was identified as *Bact. coli*.

Perhaps, most errors in differentiation occur with staphylococci. Many alleged albus strains are essentially aureus, having temporarily lost pigment-producing power. More important are variations in behaviour to gelatine, plasma and blood, which determine classification as saprococci or pyococci. Of 626 staphylococcus strains from diverse human sources, 524 were macroscopically normal, but of these nearly 3/4 lacked at least one of the " typical " characters; of 102, which were macroscopically dissociated, only 12 conformed to the definition of pyococci. Hence a classification which neglects phases because of fermentative behaviour may, like a purely serological method, mislead: if such is the liability to error in the case of staphylococci, what must it be in that of streptococci and other groups?

Prof. M. H. Dawson, Dr. G. L. Hobby and **Dr. M. Olmstead** (New York): *Variation in* Streptococcus hæmolyticus.

The present report deals with variation in the various groups of hæmolytic streptococci as defined by Lancefield. Following her classification, the phenomena of variation in Groups A, B, C, D, E, F and G have been studied. The results reveal the existence of similar variation trends in all instances. Three chief variant phases, designated by the terms Mucoid (M), Smooth (S) and Rough (R) have been identified in each group. The following attributes of each of these variant phases have been investigated: (*a*) Colonial

morphology; (*b*) Morphology of the individual bacteria; (*c*) Capsulation; (*d*) Character of growth in plain broth; (*e*) Suspension stability in salt solution; (*f*) Virulence; (*g*) Antigenic structure.

The studies indicate that the variation pattern in each of the Groups B, D, E and F is strikingly similar and closely analogous to that of *Diplococcus pneumoniæ*.

In Groups A, C and G, the pattern corresponds closely with that of the other groups in all respects save that of antigenic structure. In these groups the question of type-specificity in the Mucoid phase occupies an anomalous position. The situation has been in part explained by the demonstration that Mucoid organisms of Groups A and C elaborate soluble polysaccharides, but it has not been possible to show that these substances possess type-specificity.

Finally, the study shows that, in general, the phenomena of variation in the various groups of hæmolytic streptococci conform to the pattern exhibited by a wide variety of other bacteria.

Prof. M. H. Soule (Ann Arbor, U.S.A.): *The Nature of the Proteins synthesized by the Colon Bacillus and Agglutination Studies with* Bact. coli *cultured on Synthetic Media.*

Typical S forms of representative strains of the colon-typhoid-dysentery group were cultured under aerobic or anaerobic conditions on an agar medium containing alanine or cystine. The synthesized proteins of *B. coli* grown on the two mediums contained about the same percentage of the basic amino-acids, arginine, histidine and lysine, which would suggest that identical proteins were formed by the organisms. The second part of this study has to do with the agglutination reaction. The organisms were cultured serially through ten generations on the aforementioned media under the desired gaseous environments before making the antigens. The cells were washed twice with saline, then suspended in saline, after which they were exposed to 56° C. for one hour, and subsequently standardized as to cell count. The antigens thus prepared were injected subcutaneously and intravenously into rabbits. Following a definite incubation period the animals were bled and the sera gathered. An extensive series of agglutination tests were carried out, employing homologous antigens as well as preparations made from typical R and M types of *B. coli* cultured aerobically on plain infusion agar. It was not possible to maintain the colonial characteristics of the R and M types when they were grown on synthetic media. All tests were carried out by adding the germ suspensions to the serum dilutions. The tubes were placed in the water bath for one hour at 45° C. and subsequently stored at 15° C. for 24 hours before reading. The antisera had very low titres against their homologous organisms, but considerably higher titres versus the cells grown on plain infusion agar. Our data indicate that serologically the colon bacillus has the same antigenic structure

when cultured on synthetic mediums containing alanine or cystine under aerobic or anaerobic conditions.

Prof. I. C. Hall (Denver, U.S.A.) : *Metabolic " Mutation " in the Genus* Bacterium.

The frequency of metabolic " mutation " among the non-sporulating Gram-negative rods of the intestinal tract, here allocated to the genus Bacterium, and particularly the common occurrence of *B. coli mutabile* (Massini) in human fæces, in wound infections, in foods involved in outbreaks of gastro-enteritis, and in water supplies, together with the possible confusion of this organism with *B. paratyphosum*, are emphasized.

The criteria of metabolic mutation are—

(1) Slow fermentation of a certain carbohydrate ;

(2) Formation of multiple papillæ or numerous secondary daughter colonies in primary colonies grown upon solid media containing the significant carbohydrate ;

(3) The isolation of a pure non-reverting culture of the " mutant " which rapidly ferments the significant carbohydrate, but does not produce multiple papillæ, or daughter colonies, upon solid media containing this carbohydrate.

The occurrence of metabolic mutation in relation to lactose is again recorded for *B. cloacæ* (Jordan), *B. dispar* (Andrewes), and *B. dysenteriæ* (Sonne) as well as independent " mutation " in relation to other carbohydrates in *B. coli mutabile.* The forms of colonies of all of these were illustrated by lantern slides.

The simultaneous, but apparently unrelated R and S dissociation in certain strains, was likewise discussed briefly.

Dr. M. Levy-Bruhl and **Mlle. Yvonne Cado** (Paris) : *Phénomènes de mutation observés dans le groupe des bacilles muqueux (Pneumobacille, Lactis-Aerogenes).*

Certains auteurs (Julianelle, Legroux) ont signalé des variations spontanées ou provoquées dans les caractères présentés par des échantillons de bacilles muqueux conservés *in vitro.* Ces modifications portaient sur l'aspect des colonies qui peuvent revêtir le type " Rough " ou " Smooth."

Nous mêmes avons publié, en 1934, l'observation d'un cas de chromogénèse apparue dans une souche de Pneumobacille conservée dans notre collection depuis 18 mois.

Nous constations la production d'un *pigment jaune d'or* très abondant, coexistant avec la disparition de certaines propriétés caractéristiques du Pneumobacille.

Depuis lors, nous avons eu l'occasion d'observer un nouveau cas de chromogénèse apparue dans une souche de collection ; le

No. 418 de l'Institut Lister ; mais cette fois il s'agit d'un *pigment rouge* et nous n'avons pu l'obtenir, jusqu'à présent, que sur la pomme de terre ; par ailleurs tous les autres caractères du germe sont restés typiques : c'est donc un véritable pneumobacille chromogène.

D'autre part, en vérifiant périodiquement les caractères présentés par nos souches, nous en avons trouvé quatre qui, en culture très jeune (alors qu'elles étaient primitivement immobiles) ont montré des germes doués de mouvements actifs très nets ; cette *mobilité* comme nous avons pu le constater avec l'aide du Dr. Levenson est liée à la présence de cils qui disparaissent, en même temps que les mouvements, dans les cultures plus âgées.

Un autre caractère nouveau est apparu dans ces mêmes souches : c'est un *pouvoir protéolytique* faible, mais net vis à vis de la gélatine et sans action sur le sérum coagulé. Par ailleurs ces échantillons présentent toutes les autres propriétés du pneumobacille. Il semble s'agir d'une évolution vers le type *Aerobacter-Cloacæ* (Jordan).

Ces phénomènes de mutation ne paraissent pas encore avoir été signalés dans ce groupe bactérien : cependant Krauss, 1926, avait observé une souche de bactérie muqueuse, protéolytique et chromogène présentant une variabilité de caractères telle qu'il avait dénommé ce germe : *Bactérium mucosum mutabile.*

Prof. A. Rochaix and **Dr. P. Rivollier** (Lyon) [Communicated by Dr. A. R. Prévot (Vanves, France)] : *Sur une nouvelle dissociation du staphylocoque.*

En cultivant des staphylocoques dorés en bouillon T (Truche, Cramer et Cotoni), on obtient au bout d'un certain nombre de générations, des cultures constituées par de petits grumeaux, nageant dans un liquide plus ou moins clair. Ensemencées sur gélose ordinaire ces cultures donnent des colonies de deux types. les unes petites, opaques et présentant un pigment jaune plus ou moins foncé ou doré (type A) ; les autres plus grandes, très blanches et comme porcelainées (type B).

Les staphylocoques du type A se présentent en cultures constituées par des grumeaux nageant dans un liquide clair et se déposant rapidement au fond du tube. Ceux du type B donnent des cultures très homogènes, avec léger voile à la surface et petit depôt blanc dans le fond du tube. Du point de vue morphologique et de la fermentation on n'observe aucune différence. Les deux types peuvent liquéfier la gélatine, mais le type A a une action protéolytique tres marquée. Le type A a une action hémolytique souvent totale ; le type B n'a qu'une action véritablement insignifiante.

Quant à l'agglutination par les sérums de lapins normaux, ou les immunsérums provenant soit du type A, soit du type B, il se produit une agglutination spontanée (tubes témoins) qui ne permet guère d'apporter de précisions, mais il semble que les immunsérums

préparés avec le type A, favorisent agglutination des souches de ce même type.

Il n'y a pas de différence entre nos deux types vis-à-vis la trypaflavine. La dissociation que nous avons obtenue est donc absolument différente de celle (S et R) de Sabatucci. L'agglutination non-spécifique par la chaleur ne nous a donné que des résultats négatifs.

Il est possible de passer d'un type à l'autre, mais alors qu'on peut transformer facilement un type A en type B il faut, au contraire pour passer de B à A un nombre considérable de repiquages en bouillon T. Du point de vue de pouvoir pathogène la différence entre nos deux types est très nette. Le type A se montre beaucoup plus pathogène que le type B. Quant à la valeur antigène, elle est plus grande pour le type B. Nous n'avons pas utilisé les dénominations de " R " et de " S," car nos types dissociés ne répondent pas aux caractères typiques. Le type A est seul à donner des colonies présentant un pigment jaune doré, alors que le type B en est totalement dépourvu. Ce fait intéressant parait résoudre la question si longtemps controversée de la nature (variété ou espèce) des staphylocoques jaunes dorés et des staphylocoques blancs.

Major H. C. Brown, Dr. J. C. Broom and **Dr. C. A. Hoare** (London) : *Variation in the Electric Charge of Trypanosomes.*

The sign of the electric charge of trypanosomes was determined in a closed microcataphoresis cell with non-polarizable electrodes. Under constant standard conditions the insect and cultural forms were negatively charged.

The blood forms, however, may be either positively or negatively charged. In some instances both positively and negatively charged trypanosomes may be present in the blood of an infected animal simultaneously.

No alteration in the sign of the charge takes place in continuous progressive infections during serial passage in mice for more than thirty generations.

When trypanosomes disappear temporarily from the peripheral blood and later reappear, the sign of the charge of the relapse variant is the opposite of that carried by the original strain.

In many instances alteration of the host has been found to change the sign of the charge in trypanosomes. In the case of *Trypanosoma evansi* the " positive " variant is considerably more susceptible to the action of tryparsamide than the " negative " variant.

Serological differences also occur in the two variants.

Dr. G. P. Gladstone (London) : *Antigenic Composition and Virulence of* Bact. typhosum *in Relation to its Growth Requirements.*

The virulent (Vi) strains of *Bact. typhosum*, described by Felix, grow easily on a purely synthetic medium containing a mixture of

glucose, amino-acids and the usual salts. On this medium the organism remains highly virulent for the mouse, even after several subcultures. It also retains its agglutinability with a pure (absorbed) Vi antiserum and its inagglutinability with a pure O antiserum.

As with other strains of *Bact. typhosum*, it is possible to train the Vi organism to dispense with amino-acids as sources of nitrogen and to utilize ammonia instead. In certain circumstances, virulence, agglutinability with Vi antiserum and inagglutinability with pure O antiserum persist throughout the process of training. In this process the removal of tryptophan presents greatest difficulty.

Growth on these media produces a temporary, complete or partial suppression of the flagellar antigen. This is regained immediately on transference to nutrient agar.

Dr. R. Th. Scholtens (de Bilt, Holland): *Acquired Resistance against Bacteriophage in Connection with the Point of Attack of the Phage in the Microbe.*

The fact that resistance obtained against one bacteriophage is no protection against lysis by other bacteriophages is explained by d'Hérelle by differences in virulence.

The following experiment, however, cannot be explained in this way.

Of two bacteriophages, namely, Ty 1, which only attack typhoid bacilli, and Ty-Gä 7, which has, amongst other things, affinity for Gärtner bacteria, the bacteriophage Ty 1 makes typhoid cultures resistant to itself but not towards Ty-Gä 7. Conversely, the bacteriophage Ty-Gä 7 makes the bacterium resistant towards itself but not towards Ty 1. This is explained by the two bacteriophages each having a different point of attack on the bacteria. The bacteriophage does not confer resistance against another bacteriophage having a different point of attack.

Other bacteriophages were isolated, four which acted only on typhoid bacilli, and three which also attacked Gärtner bacilli. Each five of the bacteriophages which attacked only typhoid bacilli (bacteriophages Ty 1, 2, 3, 4, 5) made typhoid bacilli resistant to each of these five bacteriophages, but not against the four bacteriophages attacking Gärtner bacilli. Conversely, each of the four bacteriophages Ty-Gä 6, 7, 9, 10 made typhoid bacilli resistant to each of these bacteriophages, but against none of the bacteriophages Ty 1, 2, 3, 4, 5.

This was explained by assuming that the bacteriophages Ty 1, 2, 3, 4, 5 all had the same point of attack on the bacteria, which differed from the point of attack common to the bacteriophages Ty-Gä 6, 7, 9, 10. Each of the bacteriophages Ty 1, 2, 3, 4, 5 protect the common point of attack and thereby make the bacteria resistant to each of the five bacteriophages. Likewise, the bacteriophages Ty-Gä 6, 7, 9, 10 make their common point of attack

resistant and so each bacteriophage is able to bring about resistance against each bacteriophage of this group.

Thus the incompleteness of the resistance obtained must not be explained by differences in virulence, but by different bacteriophages having different points of attack on bacteria and their being able to protect only their own particular attacking region.

The attacking region of bacteriophages Ty 1, 2, 3, 4, 5 is the Vi-receptor.

The attacking region of bacteriophages Ty-Gä 6, 7, 9, 10 is the receptor III of Bruce White.

The bacteriophages Ty 1, 2, 3, 4, 5, which presumably attack the Vi-receptor, transform the O-resistent type into the O-sensitive type. The other bacteriophages cannot do this.

Mr. P. Bruce White (London): *Resistance developed against Bacteriophage.*

The peculiarities of the resistant secondary growths arising after bacteriophagy have attracted much superficial attention, and the bacteriophage has been freely acclaimed as a powerful agent in bacterial mutation and evolution. While the belief that he may, even indirectly, determine bacterial change may flatter the conceit of the bacteriologist, there does not actually exist one whit of evidence that the peculiarities of the resistant growth are ever progressive in the sense of the evolutionist—or, indeed, that they arise under the action of the bacteriophage. Such experimental evidence as is available suggests that the resistant races are (1) loss forms (e.g., R or ρ), or (2) forms which have reverted from a sensitive reduced state to the insensitive normal state (e.g., R → S) and, further, that these forms are actually present in the culture before the bacteriophage is added, arising by faults (or reversions), during the multifold divisions of the cells.

It is moderately clear that there is nothing adaptative about the resistance of the secondary growth. The resistant secondary growth merely represents an unassailable residuum of the original culture in which the specific pabulum (be it polysaccharide or of other nature) of the phage in question is either lacking or mechanically shielded from attack.

Dr. H. Loewenthal (London): *The Colony Forms of Hæmolytic Streptococci.*

The biochemical properties, virulence, antigenic structure and pathogenicity of different colony forms of a hæmolytic streptococcus of group C have recently been studied in detail in our laboratory by Pradhan. Three colony forms were found: M the mucoid, N the non-mucoid, and O the intermediate. Subcultured, they bred true for a fairly long time, but conversion from one form to another

was possible by cultivation in immune serum. A remarkable difference in the faculty of growing in bile media could be demonstrated. The failure of one single form—M— to grow on bile seems the more significant, as this medium is frequently used for differentiating strains or even groups of streptococci. The virulence of all the three forms for mice was about the same (m.l.d. 10^{-6}–10^{-7} c.c.) and it is for this reason that I am unable to accept any terminology of variants connected with the virulence terms " S " and " R." I am of the opinion that this latter kind of variation, connected, as it is, with the loss of virulence, may occur in any of the colony forms. The three forms vary in their saline stability and in their agglutinability by acid. Experiments on the saline stability and serum agglutinability of boiled organisms revealed different heat-stable and heat-labile antigenic components. Precipitation with absorbed sera showed an additional antigen in the M form. It is to be expected that chemical analysis will lead to a better understanding of the significance of the colony forms; for instance, a simple acetic acid test distinguishes M from the other forms grown in fluid medium. Finally, a distinct difference was found in the reactions caused by the intradermal inoculation of the N form and the M form in the rabbit's ear. While the former produced a dry erysipelas-like inflammation, the M form caused a soft, rather boggy swelling, thus revealing the different pathogenicity of colony forms from a single strain.

Dr. A. Compton (Alexandria, Egypt): *Variation in* Bact. dysenteriæ alexandrinus.

The variations described by the speaker for *B. dysenteriæ alexandrinus*, a Gay-Harris group organism, which is a cause of dysentery in about 10 per cent. of acute bacillary dysenteries met with in the Middle East, lend support to the re-classification of *Streptococcus hæmolyticus* into the M, S, R colonies of Dawson, Hobby and Olmstead. The pictorial representation of colonial morphology, and morphology of the individual bacteria of the variants III, IV and VI in the cycle-scheme outlined by Compton[1] for the former organism parallels very closely the pictures shown by Dawson and collaborators for streptococci.

But the dysentery organism in question shows something more than M, S, R, variation, in that such variants appear in the light of transformation offspring of Opaque (O) and Translucent (T) precursor colonies. Indeed the O → T transformation phenomenon may be regarded as supplementary to, if not independent of, the M, S, R variation.

It is suggested that this dysentery organism, in view of the varying susceptibility to bacteriophage of the precursors O and T (both S forms), the contrast in virulence of the several variants,

[1] *Ann. Inst. Past.*, 1935, **34**, 715.

and the evolution histories of the latter, may prove of interest to workers concerned in elucidating the chemical constitution of antigens.

DISCUSSION *on* PRINCIPLES AND METHODS FOR THE QUANTITATIVE DETERMINATION OF ANTIGENS AND ANTIBODIES, INCLUDING THEIR DIAGNOSTIC APPLICATION

Chairman : Prof. R. Otto (Frankfurt a.M.).

Prof. Th. Madsen (Copenhagen), **Opener.**

(1) *Direct measuring of antigen* (determination of toxicity). Titrations based on hæmolysis or flocculation in many instances cannot yet replace animal experiments. For quantitative determinations the *quality of* the experimental animals is of enormous importance. Purchase of animals from dealers involves a risk of differences in resistance even in animals of the same weight, differences of age, and latent infections, which appear especially in the winter and spring. It is preferable to breed our own experimental animals and to give them protective food, to avoid seasonal variations of their resistance.

Intracutaneous methods should be employed as far as possible (English and American authors: Claus Jensen's intracutaneous rabbit method). Advantages: Many tests on the same animal, elimination of individual differences of sensitiveness, a much smaller percentage of error, the possibility of measuring very small amounts of toxin, and considerable economy in animals.

The relation of the dose of antigen to the result of the test is studied in such a way that the *steepness of the curve* is established. The toxin determination is carried out with doses on the steep rise *of the curve—for instance, dl 50 or dl 60 (Trevan), which ought to* replace the old " dosis minima letalis," " dosis certe letalis," etc.

(2) *The quantitive determination of the immunizing power of antigens* involves considerable difficulties, particularly in titration of diphtheria-formol-toxoid (anatoxin).

The simplest solution would be employment of the flocculation reaction. But there is no close parallelism between flocculation units and immunizing power—also the presence of substances such as aluminium hydroxide, tapioca, etc., as a rule increases immunizing power.

In experiments with titration on *animals*, the following points are to be observed:

(*a*) Comparison with a standard preparation.

(*b*) The animals must be selected, as defects will be pronounced in the long interval between injection of antigen and determination of immunity. This may manifest itself

by deviations from the normal curve of weight increase, and such animals may have to be excluded. Lowered immunizability in the winter and spring must be considered.

(c) The immunization curve must be determined. If the immunization curve is not known, there is the risk that the determination of immunity may be made sometimes in its ascending period, sometimes in its descending period—this will invalidate a comparison between antigens.

(d) *The antigen dose* is of the greatest importance. The immunity obtained is practically minimal up to a certain dose of antigen, whereafter the curve shows a steep rise till it reaches its highest level, so that even a great increase in the dose of antigen fails to produce any additional degree of immunity. To be serviceable the titration must be made with antigen doses corresponding to the rise of the curve.

(e) *The determination of immunity* is carried out most often after 4 weeks. The criterion generally employed is resistance to a high multiple of *dosis letalis* of diphtheria toxin injected intravenously. The percentage of survivals is used to measure immunity, but it requires a large number of animals.

A better utilization of animals is obtained by *antitoxin estimation* in *each* animal. As animals may differ enormously in antitoxin production (1–10000) after the same injection of antigen, it requires many animals for this estimation.

The toxin resistance appears dependent upon the antitoxin content of the blood.

(3) *Titration of antibody.* Comparison with a standard serum *ad modum* Ehrlich, adopted by the Commission on Biological Standardization of the League of Nations Health Organization, is based on the same principles as toxin estimation.

It is very important that reactions between antigen and antibody are fully completed before injection, which it is preferable to give intravenously. It is important that test antigen and test antibody are not reacting too slowly, as there is otherwise risk of inaccuracy.

Prof. H. R. Dean (Cambridge) : *Quantitative Methods in Precipitation and Complement Fixation Experiments.*

The amount and speed of formation of precipitate depend on the proportions of antigen and antibody in the mixture.

The optimal point can be determined by three methods ; in two of these the proportions of antigen and antiserum most favourable to particulation being determined, and in the third the propor-

tions yielding the greatest precipitate. The optimal points may not, and usually do not, coincide. But provided the proportions of the two reagents remain constant, irrespective of the absolute quantities of the ingredients, any of the methods can be employed for the quantitative determination of antigen or antibody, and have so been used.

The neutral point. Which, if any of the three optimal points corresponds to a neutral mixture ? Evidence was presented to show that the optimal tube of the flocculation method in which antiserum is kept constant represents the neutral point. The supernatant fluid from such a tube should contain neither antigen nor antibody while those to the left (relative antigen excess) and those to the right (relative antibody excess) should contain, respectively, antigen and antibody. Dean and Webb (1926) showed that these expectations were realized under the above conditions ; they measured the antibody excess quantitatively and found that in some, but not in all, cases the antibody excess agreed exactly with the amount to be expected on the assumption that the optimal flocculation point corresponded to the neutral point. Confirmatory results were obtained by Taylor (1931), Marrack and Smith (1931), Wilson Smith (1932) Duncan (1932) and Hooker and Boyd (1935), using a variety of antigens and their homologous antibodies. Heidelberger and Kendall (1935) also refer to the probable correspondence of the constant antibody flocculation optimum with the equivalence point.

Indirect evidence of the accuracy of the constant antiserum method is furnished by Taylor, Adair and Adair (1932), who found, by this method, 7.29 per cent. of crystalline egg albumin in egg white ; by other methods, Hopkins (1900) found 6 per cent. and Wu and Ling (1927) 8.5 per cent. Taylor, Adair and Adair also found 4.46 per cent. of globulin in horse serum by this method, while Hammersten (1878) found 4.57 and Gibson and Banzhaf (1910) 4.07 per cent. By this method, Wilson Smith (1932), using the specific polysaccharide as antigen, showed that the values obtained were a true index of their protective power as determined by independent large scale mouse protection tests. Other tests on 12 samples of sera showed that the experimental error of the method is small.

The occasional discrepancy found when the two optimum flocculation point methods (constant antiserum and constant antigen) are used is difficult to explain. In the constant antiserum method the concentration of the antiserum profoundly affects the rate of flocculation. If results by the two methods vary, the optimal tube of the constant antigen method contains a relatively higher concentration of antiserum than the optimal tube of the constant antiserum method. It seems possible that in the constant antigen method, the order of particulation is determined largely by the proportions of the ingredients and also by the varying concentrations of antiserum.

In some, but not all, cases as observed by Brown (1935) the precipitate particles at the optimal zone, unlike those of the outlying ranges, resist disintegration on shaking.

Mr. A. T. Glenny (Beckenham, England): *Some Anomalies in the Titration of Antigens and Antibodies.*

Many anomalies are encountered in the titration of antibodies and antigens; reliance should not be placed upon any single method of testing.

The flocculation value of non-avid (loosely combining) diphtheria antitoxin is greater than the *in vivo* value; a greater *in vivo* value indicates the presence of non-flocculating antitoxin, which, however, combines with toxin *in vivo*.

Multiple toxins and antitoxins are revealed by differences in hæmolytic titration using sheep and rabbit cells (Staphylococcus) or by differences between hæmolytic, necrotic and lethal action (*Cl. welchii* of different types).

The titration of tetanus antitoxin varies with the toxin used, because official standard sera are non-avid. The apparent combining power determined against these standards varies with some quality (possibly degree of affinity) of the toxin.

A fractional dose of any toxin requires a relatively smaller fraction of avid antitoxin for neutralization owing to the reduction of indicating doses present, but relatively more non-avid antitoxin owing to dissociation of the toxin-antitoxin complex on dilution.

Fractional doses of *Cl. welchii* α toxin require more antitoxin for neutralization in the presence of certain normal sera, because some sera increase the hæmolytic power of the toxin more than tenfold.

Some preparations of diphtheria toxoid-antitoxin floccules appear far weaker antigens than toxoid-antitoxin mixtures or toxoid when judged by the response of guinea-pigs to a single injection of 1.0 or 5.0 c.c., but appear stronger when tested by two spaced injections of 0.1 or .01 c.c.

Diphtheria toxin partially modified by formalin, appears more toxic when injected intracutaneously in large than in small guinea-pigs, although the guinea-pigs are almost equally susceptible to dilutions of unmodified toxin.

Certain toxins (*Cl. welchii*, Wilsdon Type "D") increase in toxicity when kept in phosphate buffer solution.

Diphtheria toxoids of the same flocculating value but, prepared under different conditions, vary greatly in antigenic efficiency.

Dr. G. Ramon (Garches): [Communicated by Prof. M. Weinberg (Paris)]: *La méthode de floculation et le dosage des antigènes et des anticorps. Principes—Résultats.*

La méthode de floculation a trouvé son origine dans le *phénomène de floculation* mis en évidence en 1922 dans les mélanges de toxine

diphtérique et de sérum antidiphtérique (G. Ramon). Elle est basée sur l'apparition de la "*floculation initiale*" qui indique à l'expérimentateur le mélange dans lequel la toxine et l'antitoxine spécifiques se sont saturés mutuellement et aussi exactement que possible (*mélange optimum*). Elle est très généralement employée à l'heure actuelle pour le dosage de certains antigènes et anticorps : toxine, antitoxine diphtériques, etc.

La méthode de floculation et son principe peuvent être appliqués au dosage des précipitines en général (G. Ramon 1923). Cette application a été effectivement réalisée en ce qui concerne le dosage des précipitines sériques (Dean et Webb 1926—Taylor, etc.).

La méthode de floculation permet d'apprécier la "*valeur intrinsèque*" de certains antigènes tels que la toxine, l'anatoxine diphtérique, par exemple, valeur qui mesure l'activité immunisante de ces antigènes. Elle s'est révélée, en cela, nettement supérieure aux méthodes *in vivo*. En effet, le pouvoir immunisant d'un antigène, par exemple de l'anatoxine diphtérique déterminé chez un animal est *relatif* à cet animal. Il dépend de son aptitude à s'immuniser, aptitude qui varie d'une espèce à l'autre, d'un individu à l'autre, qui varie dans certaines conditions encore (existence possible chez les animaux d'une immunité naturelle occulte, par exemple en matière d'immunité antistaphylococcique). Grâce à la méthode de floculation le titre de l'antigène est ainsi établi uniquement *en fonction de l'anticorps*, il exprime bien la puissance intrinsèque de l'antigène de se combiner à l'anticorps et d'en engendrer, *in vivo*, la production.

L'importance de la méthode de floculation s'est affirmée en particulier par les progrès qu'elle a facilités, au cours de ces dernières années, en ce qui concerne l'obtention de toxines et d'anatoxines (diphtériques par exemple) de valeur antigène intrinsèque élevée (G. Ramon, Glenny, S. Schmidt, etc.).

Prof. R. Prigge (Frankfurt a.M.) : *Theorie und Methodik der Antigenmessung.*

Die Theorie der Messung von Antikörpern ging von der unausgesprochenen Voraussetzung aus, dass die *Tiere einer bestimmten Spezies auf gleiche Reize annähernd gleichartig reagieren.* Diese Voraussetzung fand ihre Bestätigung durch die Erfahrung, dass eine genau bemessene Serummenge sämtliche Versuchstiere gegen eine beliebige Dosis gewisser Gifte zu schützen vermag, während bereits eine etwas geringere Serummenge regelmässig versagt. Es ist also ohne weiteres möglich, eine Reihe von Seren mit Hilfe des "einfachen Reihenversuches" sehr genau zu *titrieren.*

Seit langem ist es allerdings bekannt, dass die Versuchstiere nur annähernd gleichartig reagieren und dass gewisse *individuelle Unterschiede* zwischen ihnen bestehen. Ein nennenswerter Fehler ist jedoch bei der Anwendung des "einfachen Reihenversuches"

nicht zu erwarten, wenn die Grenzdosen, welche für *alle Tiere* wirksam bzw. unwirksam sind, sich nur wenig unterscheiden. *Prigge* u. *Hartoch*[1] konnten zeigen, dass der Fehler höchstens + 11 Proz. bzw. — 10 Proz. des wahren Wertes beträgt, wenn die beiden Grenzdosen nur um 10 Proz. auseinanderliegen. Sie wiesen aber nach, dass *der Fehler des einfachen Reihenversuches ausserordentlich gross wird, wenn die Spanne zwischen den erwähnten Grenzdosen zunimmt.* Man kann unter diesen Umständen Überleben oder Tod des *einzelnen Versuchstieres* nicht mehr als Indikator für einen bestimmten Wert verwenden. Um reproduzierbare Ergebnisse zu gewinnen, muss man vielmehr das Verhalten von *Tier-Kollektiven* prüfen.

Auch bei der Messung der Wirksamkeit von *Antigenen* liegen die Verhältnisse so ungünstig, dass der einfache Reihenversuch durchaus versagt. Auf Grund eines Prinzips, das zuerst von K. Kisskalt[2] entwickelt worden ist und dessen Anwendbarkeit auf die vorliegenden Probleme ich an anderer Stelle [3,4] eingehend dargelegt habe, habe ich die Tiere einer grossen Frankfurter Tierfarm nach der Menge der zu ihrer Immunisierung erforderlichen Antigendosen in *Klassen* eingeteilt und diese in der aus der Vererbungsforschung allgemein bekannten Weise graphisch dargestellt. Zur graphischen Darstellung der Abszissen wurde nach dem Vorschlag von Trevan[5,6] ein logarithmetrischer Masstab benutzt, bei dessen Anwendung die Resultate sich durch sogen. "*normale Verteilungskurven*" wiedergeben lassen. Diese Untersuchungen liessen das überaus verschiedenartige Verhalten der Frankfurter Tiere sehr deutlich erkennen. *Zur Immunisierung der "lässigsten" Tiere ist etwa 30,000 mal so viel Antigen notwendig wie zum Schutz der reagibelsten Tiere. Unterschiede in der Wirksamkeit sind daher bei einem Tiermaterial, wie es mir in Frankfurt bisher zur Verfügung stand, erst dann wahrnehmbar, wenn die untersuchten Antigenmengen ausserordentlich verschieden sind oder wenn zu den Untersuchungen eine sehr grosse Anzahl von Tieren verwendet wird.* Die Genauigkeit von Antigenmessungen ist unter diesen Umständen sehr beeinträchtigt.

Die zu meinen ersten Untersuchungen verwendeten Tiere waren *ohne Rücksicht auf genetische Prinzipien* gezüchtet. Um die Frage zu prüfen, ob die *individuellen Schwankungen der Immunisierbarkeit* bei einem erbbiologisch homogeneren, möglichst *reinlinigem Tiermaterial* geringer seien und ob die bei der Messung von Antigenen erzielten Resultate durch Verwendung derartiger Tiere verbessert werden könnten, prüfte ich eine Reihe von Meerschweinchen-Stämmen,

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1 *Arb. a.d. Staatsinst. f. exp. Therapie u.d. Georg-Speyer-Hause*, 1930, 23, 1.
2 *Ztsch. f. Hyg.*, 1915, 81, 42.
3 *Arb. a.d. Staatsinst. f. exp. Therapie u.d. Georg-Speyer-Hause*, 1935, 23, 18.
4 *Zbl. Bakt. I. Orig.*, 1935, 135, 32.
5 *J. Path. & Bact.*, 1929, 32, 127.
6 *Proc. Roy. Soc. B.*, 1931, 108, 54.

die der Göttinger Zoologe F. Kröning durch jahrelang fortgesetzte *Bruder-Schwester-Inzucht* gewonnen hatte und die zum Teil einen beträchtlichen Grad von *Gleicherbigkeit* erreicht hatten. Hierbei ergaben sich *ganz ausserordentliche Unterschiede* zwischen den untersuchten Meerschweinchenstämmen. Die Stämme verhalten sich nicht nur in ihrer *mittleren Immunisierbarkeit,* d.h. in der Höhe der zur Immunisierung von 50 prozent der Tiere erforderlichen Antigendosis, sondern auch in ihrer *Variationsbreite* ganz verschieden. Die extremen Varianten verschwinden bei den ingezüchteten Stämmen, und die Besetzung der Mittelklassen nimmt zu. Das Verhältnis der Dosen, die zum Schutz der " reagibelsten " bzw. der " lässigsten " Tiere erforderlich sind, beträgt beim :

Frankfurter Tiermaterial	=	1 : 32000
Inzucht-Stamm XX	=	1 : 14000
Inzucht-Stamm XI	=	1 : 2000
Inzucht-Stamm XXX	=	1 : 100
Inzucht-Stamm XXIIa	=	1 : 25

Die Bedeutung dieser Ergebnisse ist leicht zu erkennen. Zu Messungen, die mit dem Stamm XXIIa durchgeführt werden, sind infolge der verhältnismässig geringen Unterschiede in der Immunisierbarkeit der einzelnen Individuen *sehr viel weniger Tiere* erforderlich als zu Messungen, zu denen das Frankfurter Tiermaterial verwandt werden muss.

Zum Schluss sei noch auf eine besonders wichtige Tatsache hingewiesen. Wie die Untersuchungen von Madsen und seinen Mitarbeitern über die Saisonschwankungen der *Antikörperbildung* erwarten liessen, gibt es neben den individuellen Unterschieden in der Immunisierbarkeit auch sehr beträchtliche *zeitliche Unterschiede im Verhalten des Gesamttiermaterials,* ganz gleichgültig, ob es sich um ingezüchtete Tiere handelt oder nicht. Stets ist im Winter eine sehr viel grössere Antigenmenge zur Immunisierung der Tiere erforderlich als im Sommer. Ausserdem konnte ich Schwankungen beobachten, die nicht saisonbedingt sind, sondern sich über *mehrjährige Perioden* zu erstrecken scheinen. Wie ich bereits anlässlich der im Sommer 1931 abgehaltenen Londoner Diphtherie-Konferenz nachdrücklich betont habe, ist eine Überwindung der durch die Instabilität des Tiermaterials bedingten Schwierigkeiten und eine exakte Durchführung von Antigenmessungen nur dann möglich, wenn man das *Tiermaterial für jede einzelne Untersuchung mit Hilfe eines Standardpräparates eicht*[7].

Die Prinzipien, welche bei der Wertbemessung von Diphtherieimpfstoffen berücksichtigt werden müssen, gelten auch für die biologische Messung anderer Antigene. Ihre Anwendung hat sich vor allem bei der Bewertung von Impfstoffen, die der aktiven Immunisierung gegen *Shiga-Kruse-Ruhr* dienen, als fruchtbar erwiesen.

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[7] *League of Nations. Health Organization,* 1931, 1056 (i), 8.

Die ausführliche Veröffentlichung dieses Vortrages erfolgt in der "Zeitschrift für Hygiene" Bd. 119; H. 2; S. 186.

Dr. L. D. Felton and **Dr. Helene J. Stahl** (Baltimore, U.S.A.): *Methods of Assay of Antipneumococcus Horse Sera and Concentrates.*

For assay of antipneumococcus serum and concentrate, comparison is made between a mouse-protection method and what is termed a combining equivalent technique. In both tests antibody content of an unknown serum or concentrate is estimated by comparison with a standard control serum. The mouse method consists of titration of an unknown sample and the control serum simultaneously in a strain of mice of known resistance to pneumococcus and against a standardized culture. The culture is standardized so that 50 per cent. survival of the mice occurs with a dose of antibody which lies between the concentrations showing direct proportionality between antibody and organism. In terms of units this relationship is found to be in a very narrow zone varying somewhat with different strains of organisms, but generally extending from 1 unit to 0·25 unit. In the comparisons reported below, 0·5 unit has been used throughout.

The combining equivalent method is a quantitative adsorption technique. It consists of two steps: first, mixture of constant amount of SSS with amounts of serum antibody varying by a 1 per cent. volume increment; these mixtures are incubated at 37° C. for two hours and chilled overnight at 4° C. Second, after collecting the precipitates in a centrifuge, to the clear supernatant fluids is added an excess of immune serum to measure residual SSS; the mixtures are incubated for two hours at 37° C. and then without further shaking are allowed to stand at room temperature for two hours when the endpoint is noted. The endpoint is considered as the first tube in which there is no disc precipitate. The unit content in the unknown is then found by inverse ratio by comparison with the control.

The correlation of the combining equivalent with the mouse protection results in 62 samples of concentrate is: $r = 0{\cdot}94$ for Type I, and 0·91 for Type II.

Dr. J. W. Trevan (Beckenham, England): *Errors of Sampling in the Standardization of Sera, with Special Reference to Antipneumococcus Sera.*

The statistical analysis of a long series of comparisons of antipneumococcal sera on mice was discussed. The magnitude of the errors introduced by the variability of the response of individual mice was pointed out. The bearing of these figures on other methods was dealt with. Apparent variations in the frequency distribution of the protective doses of different samples of antipneumococcal sera was discussed.

Dr. A. Eichhorn (Pearl River, U.S.A.): *International Biological Standards for Veterinary Use.*

The importance of standard biological preparations for veterinary use must be recognized not only from an economical standpoint but also for public health reasons, many of the diseases affecting animals being transmissible to man. Investigations on definite standards for veterinary biological products have been very limited, whereas the Permanent Commission on biological standardization, of the Health Organization of the League of Nations, has 27 preparations listed for which an international standard has been established.

The establishment of similar international veterinary biological standards is of utmost importance. It would be of a great advantage to have the same or a similar Commission include within their sphere of activity the assaying of veterinary products. They could further collect data on the value of the various preparations used for the control of individual diseases and thereby eliminate many products of questionable value.

The discussion covers the necessity for and advantages which would accrue from international standards of veterinary biological products, including anthrax, hog cholera, infectious bovine abortion, rabies, gangræna emphysematosa (blackleg), canine distemper, other virus diseases and diagnostic agents. The standardization of veterinary biological products would assist in safer international traffic in livestock. Another point which is of international importance is the danger existing in diseases of animals communicable to man.

The creation of an international committee is suggested for the purpose of developing international veterinary biological standards. While it is realized that it is not within the sphere of this Congress to adopt resolutions on this very important and much-neglected phase of disease control, it is hoped that an interest will be created in the minds of those attending the Congress and interested in animal disease control, to stimulate activities along this line.

Prof. M. Heidelberger, Dr. F. E. Kendall and **Mr. E. A. Kabat** (New York): *The Development of Absolute Quantitative Methods for the Micro-determination of Precipitins and Agglutinins.*

Conditions have been established for the quantitative micro-determination of the maximum amount of specifically precipitable nitrogen in antisera. After precipitation with a slight excess of antigen or hapten the nitrogen in the washed precipitate is determined by the micro Kjeldahl method, giving, after deduction of antigen or hapten nitrogen precipitate, a result in absolute terms, i.e., mg. of antibody nitrogen per ml.

The course of the precipitin reaction may be expressed quite accurately by the following expression, derived from the mass law :

mg. antibody nitrogen precipitated $= 2\,RS \dfrac{-R^2S^2}{A}$; in which R is the ratio between antibody nitrogen and antigen or hapten at a definite reference point in the "equivalence zone," S is mg. of antigen or hapten added, and A is the amount of antibody precipitated at the reference point. This equation permits the characterization of an antiserum with a minimum number of analyses.

The method has been used to show the parallel between the precipitin content of Type I antipneumococcus sera and mouse protection, for following quantitatively the development of antibody in rabbits, for the determination of small amounts of haptens or antigens, for the determination of the albumin and globulin content of normal and pathological human sera, and for an investigation of the mechanism of the precipitin reaction which is still in progress.

By nitrogen determinations on measured quantities of bacterial suspensions and on similar suspensions agglutinated by appropriate antisera these methods have been extended to a study of the agglutination reaction and to the absolute determination of agglutinins in antipneumococcus sera. Antibodies to R and S (Dawson S and M) strains may be accurately measured, and the presence or absence of type-specific polysaccharide determined. By this method pneumococcus I anticarbohydrate precipitin and agglutinin have been shown to be identical.

Dr. R. A. O'Meara (Beckenham, England): *Quantitative Determination of Antigens in Relation to Optimal Methods of Antigen Preparation.*

The use of Lf, Lr, L+, L_H, etc., is common for investigation of exotoxin production, and the principle of the method is also applicable to study of intracellular antigen production.

Requirements :—

(1) Method of titrating antigen accurately, (2) Method of dissolving organisms completely.

Means are then available for determining effect of physical and chemical environment on the production of antigen studied.

Main factors to be determined are :

(*a*) Physical :—pH, rH, temperature, time.

(*b*) Chemical :—Energy sources, nitrogen sources, salts and metals.

Illustration was made by reference to pneumococcus.

Dr. A. A. Miles (London): *Antigenic Analysis of Bacteria by the Method of Optimal Proportions.*

The constant-antigen titration, used to find optimal reacting proportions of precipitin and antigen, can be applied to agglutinating systems. In a simple ideal system, consisting of an antiserum

containing one type of antibody and a bacterium whose surface presents a single corresponding antigen, the serum dilution particulating most rapidly with a standard suspension represents the maximum amount of antibody that can be taken up by the active groups on the bacterium, without the inhibitory effects of excess antibody taking place. Antibody in excess of this amount is incompletely absorbed. Comparison of optimal serum concentrations reacting with standard suspensions of different bacteria will offer a measure of the amount of active antigenic groupings on the bacterial surfaces, in such a simple system.

In the majority of the agglutinating systems, there are a number of antigens and antibodies, in proportions which vary from culture to culture and certainly vary from one serum bleeding to another. Consequently measurements of the serum dilution or of antibody nitrogen, reacting with optimal or massive doses of bacterial suspension, yield no figure that can be translated in terms of a particular serum antibody or bacterial antigen, for in one such mixture a variety of antigens and antibodies are reacting, some in optimal proportions, and others in systems of antigen or antibody excess. Nor can qualitative differences between proportions of antigen on different bacterial surfaces, such as exist in the *Brucella* group, be measured directly by the optimal proportions method. However, by measurement of residual agglutinins left, after removal of the agglutinated mass, in the mixture used in the determination of a particulation-velocity curve, using homologous and heterologous suspensions and those of their variants as test antigens, it can be shown that the general optimum given by a curve such as is produced, for example, by a *Brucella melitensis* serum dilution series with its homologous suspension, can be regarded as a summation of at least three systems; namely, the optimal interaction of "rough" Brucella antigen with its antibody in high serum concentration, of *Br. abortus* antigen and antibody in the moderate serum concentration, and of *Br. melitensis* antigen and antibody in low serum concentration. This hypothesis is supported by the results of selective absorption of agglutinins from such a serum by preparations of specific soluble substances extracted from Brucella strains.

The optimal proportion method of analysis has an immediate application in the serology of the *Brucella* group. Absorption of a *Br. melitensis* serum by a "smooth" *Br. abortus* strain at the optimal reacting concentrations of these two reagents, or the analogous absorption of a *Br. abortus* serum by a *Br. melitensis* strain, yields diagnostic, mono-specific sera for *Br. melitensis* and *Br. abortus* respectively.

Miss Frances L. Clapp (Pearl River, U.S.A.): *The Quantitative Use of the Neufeld Reaction.*

The Neufeld reaction for typing pneumococci has been adapted for quantitative use by incubation of mixtures of pure culture with

varying dilutions of specific anti-serum. This technique has been successfully used in the titration of unconcentrated and of concentrated antipneumococcic rabbit and horse sera for Types I, II, VII, and VIII. Potency estimations thus obtained have in general paralleled those obtained by the mouse protection test. This method has also been used for the estimation of the antibody content of the sera of human beings passively or actively immunized against pneumococci.

By the use of the same technique it has been found possible to obtain capsular swelling reactions of Types I and III meningococci when mixed with specific antisera and to show great variation in the degree to which such antisera can be diluted, and still give the specific reaction. Preliminary experiments indicate that potency estimations obtained by this method may run parallel to those obtained by the mouse protection test, as in the case of anti-pneumococcic sera.

The suggestion is made that capsular swelling reactions with specific antisera may perhaps be obtained with organisms other than the pneumococci and the meningococci.

DISCUSSION *on* BLOOD GROUPS AND ORGAN SPECIFICITY

Chairman: Prof. Th. Madsen (Copenhagen).

Prof. O. Thomsen (Copenhagen), **Opener.**

1. Blood-typing (blood-grouping) of man is based on differences in the composition of the *antigen mosaic* (chiefly haptenes) in erythrocytes and, to some extent, also in other cells of the body and fluids (blood plasma (serum), saliva, urine, gastric juice, bile, etc.).

2. The composition of the antigen mosaic depends on hereditary elements (genes), most of which are active also in heterozygotic representation (less correctly called " dominants "). The hitherto established genes appear together in groups (multiple allelomorphism) or pairs. Two allelomorphic groups are known as the basis respectively of the *OAB-system* (Bernstein) and the *MN-system* (Landsteiner and Levine), besides single genes: *P* (Landsteiner), G, H (Schiff), *X* (Andreasen), etc., which are for the present to be interpreted as alternatives, i.e., presence or absence. The two allelogroups are located in different pairs of chromosomes. No instance is known of linked genes, nor of linkage between blood-type genes and other genes.

3. The *OAB-system* comprises at least four—or probably five—hitherto established genes: O(R), A_1, A_2 and B, and presumably also the recently demonstrated A_3 (Friedenreich). The three A-genes are closely related as to their effect but otherwise

independent and ranking with O and B (O. Thomsen and collaborators); possibly they are to be interpreted as different quantities of the same gene, their strength decreasing in succession.

The *MN-system* comprises at least three genes: M, N, N_2. N_2 seems to be related to N like A_2 (and A_3) to A_1 (observations by Crome and, especially, by Friedenreich). The N_2 character may be developed so faintly that it is not definitely demonstrable with the technique employed hitherto.

4. The A- and B-antigens are always inherited as units, but they are composed of some elements of differing specificity. In the various animal species components of A and B are present as species or group characters. The difference between A_1- and A_2-antigens is due essentially to a difference with regard to F-antigen* ("heterogenetic" antigen), which is represented more strongly in A_1 than in A_2. The O-gene is possibly a blank (absence of A and B), but the O character is "positive," depending upon a special antigen O-substance that *may* be developed in *all* types (at any rate in the new-born), but it is strongest in the O and A_2 type. Many findings suggest that the "extra agglutinin" α_2 is not aimed at the A_2 receptor (antigen), but is identical with anti-O, which is found in a few animals, especially among rabbits and cattle.

5. A hypothesis† is advanced about the origin and distribution of the isoantibodies in the OAB-system and the complete absence of isoantibodies in the MN-system and for most of the solitary receptors known so far. Very likely the isoantibodies, which are to be interpreted in analogy with other "natural" or "spontaneous" (dominant ?) antibodies, are dependent upon specific (dominant ?) genes of very common occurrence. The reciprocal distribution of antigen and antibody may *either* be due to the circumstance that the production of antibody becomes only manifest where the corresponding antigen does not bind it "intravitally," *or* that equipment of the organism with an antigen prevents a production of the homologous antibody. For several reasons the latter possibility seems the more probable, and the antigen development will then be epistatic in relation to the hypostatic antibody production.

The category "natural" antibodies is to be limited more narrowly than previously as it is found that many of the antibodies regarded as "natural" can be due to exogenous immunization (e.g., latent infection with diphtheria, yellow fever, etc.; occurrence of different antigen components in numerous bacterial species, food articles, etc., resulting in the appearance of amboceptor for sheep blood).

* F_A is used to designate the F-antigen specific to man with A-gene (a part of the F-complex in sheep erythrocytes).

† The writer has felt obliged to modify his view concerning the origin and distribution of the isoantibodies; *now* the formulation here advanced seems most satisfactory.

As to more recent investigations concerning the *chemical nature* of the blood-type substances, their occurrence in *various organs and body fluids*, together with a discussion of the *ability and inability to secrete* the type-specific substance, the so-called *blood-type enzyme* (Schiff and collaborators) as well as the *anthropological* and *medico-legal* aspects of the subject, I regret that time does not allow me to discuss these questions.

Prof. H. Sachs (Heidelberg): *Zur Frage der gruppen- und organspezifischen Reaktionsfähigkeit alkoholischer Gewebsextrakte und zum Blutgruppenproblem.*

Bei der Analyse der Organ- und Gruppenspezifität, zumal unter Verwendung alkoholischer Gewebsextrakte, verdienen die Abhängigkeit der Antigenfunktion von der Antigenstruktur und die Zerlegung der Antigene in Partialantigene besondere Beachtung. In ersterer Hinsicht gehört zu den wichtigsten Fragenkomplexen das Problem der Maskierung. Unter den Begriff der *Maskierung* ist auch die Hemmung der Antikörperreaktionen durch Serum als solches einzureihen. Bei hinreichender Serumkonzentration wird die Komplementbindung, aber nicht die Flockung gehemmt (Eagle, Niethammer). Dadurch wird das Verständnis für Divergenzen zwischen den beiden Ausdrucksformen der Antikörperreaktionen geebnet. So erklärt sich die stärkere Empfindlichkeit der Flockungsreaktionen bei der Serodiagnostik der Syphilis. Stark reagierende Patientensera können in kleineren Mengen bei der Komplementbindung angeben als bei der Flockung. Schwach reagierende Sera dagegen können in verhältnismässig starker Serumkonzentration flockend wirken, bei der Komplementbindung aber versagen.

Es ergibt sich daraus in vielen Fällen die Notwendigkeit der Verwendung antikörperreicher Immunsera. Unter Berücksichtigung dieser Bedingungen liess sich erneut gegenüber Angaben von Wadsworth und Maltaner feststellen, dass dem Cholesterin zweifellos Antigenfunktion zukommt (Hahn und Hazato). Gerade unter Berücksichtigung des Moments der Serummaskierung finden die von Wadsworth und Maltaner erhobenen Befunde eine Erklärung, ohne gegen das Vorhandensein von Cholesterinantikörpern zu sprechen.

Für die Frage der *Maskierung* alkohollöslicher Haptenfunktionen *durch andersartige Lipoide* sind neuere Untersuchungen von Hazato erwähnenswert. Sie liefern zugleich einen interessanten Beitrag über die Bedeutung der kolloidalen Struktur. Zellen- und Gewebsextrakte können nicht nur durch Cholesterin, sondern auch durch Lecithin in ihrer Haptenfunktion verstärkt werden. Lecithin bewirkt zugleich eine Maskierung der Haptenfunktion. Es hängt wesentlich von der Verdünnungsart ab, welcher Effekt zum Ausdruck gelangt. So bewirkt Cholesterin-

zusatz optimale Verstärkung bei rascher, Lecithinzusatz bei fraktionierter Verdünnung.

Was die *organspezifische Funktion des Zentralnervensystems* anlangt, so verdienen zwei neuere Feststellungen besondere Erwähnung. Die eine betrifft die Vielheit der organspezifischen Hirnstrukturen. Sie sind durch verschiedene Löslichkeit (in kaltem und heissem Alkohol) gekennzeichnet und stellen unabhängige Eigenschaften mit übereinstimmender organspezifischer Funktion dar (Sachs und Schwab). Dieses merkwürdige Verhalten lässt weitere Untersuchungen wünschenswert erscheinen. Im übrigen ist dem Gehirn im Gegensatz zu den Angaben von Lewis eine ausgesprochene Organspezifität zuzuschreiben (Krüpe). Auch Hirn und Hoden lassen sich durch Hirn-Antiseren im allgemeinen scharf abgrenzen, und die positiven Differenzierungsversuche sind entscheidend.

Nicht in gleicher Weise durchsichtig liegen die Bedingungen bei der *serologischen Spezifität des Geschwulstgewebes.* Die Frage der *Disponibilität* der Lipoidantigene spielt hier eine besonders bemerkenswerte Rolle, insbesondere seitdem d'Alessandro und Gaglio gezeigt haben, dass spezifische Charaktere durch Differenzen der Disponibilität bezw. durch Störung des Fettstoffwechsels vorgetäuscht werden können. Trotzdem ist die serologische Spezifität des Krebsgewebes auch unter Berücksichtigung der von d'Alessandro und Gaglio benutzten Kautelen sichergestellt.

Die Zerlegung der Antigene in Partialantigene kommt in neuerer Zeit in der *Blutgruppenlehre* zu immer deutlicherem Ausdruck. Besonders bemerkenswert erscheinen in dieser Hinsicht die neueren Studien von Thomsen über die mosaikartige Zusammensetzung der A-Eigenschaft beim Menschen und beim Kaninchen, insbesondere ihres heterogenetischen Anteils. Allerdings dürfte der Unterschied zwischen A_1 und A_2 nicht allein in der heterogenetischen Komponente gelegen sein. Dafür sprechen schon ältere Versuche von Hahn, nach denen vom Meerschweinchen gewonnene A-Antisera, in denen heterogenetische Antikörper nicht anzunehmen sind, trotzdem eine Unterscheidung von A_1 und A_2 mittels Komplementbindung gestatten.

Beobachtungen über besondere Schwäche in der Nachweisbarkeit des Merkmals A bezw. über eine Untergruppe A_3 und des Faktors N_2 (Friedenreich, Crome, Fischer und Hahn) weisen darauf hin, dass auch innerhalb der einzelnen Merkmale und Faktoren weitere Möglichkeiten der Differenzierung bestehen. In dem von Fischer und Hahn beschriebenen Fall von auffallender Schwäche der gruppenspezifischen Reaktionsfähigkeit bei Blutgruppe A, dem augenscheinlich die Untergruppe A_3 von Friedenreich entspricht, war der starke Gehalt an Iso-Antikörpern und besonders die leichte Absprengbarkeit der an A_3 gebundenen Iso-Agglutinine bemerkenswert. Offenbar kann durch Absprengung im allgemeinen umso mehr Antikörper wiedergewonnen

werden, je schwächer das Bindungsvermögen bezw. die Agglutinabilität der roten Blutkörperchen ist. Dadurch gewinnt das Absprengungsverfahren für den Nachweis schwach avider Untergruppen eine praktisch-methodologische Bedeutung.

Schwieriger zu beherrschen sind natürlich derartige Deffekttypen bei der Faktorenanalyse. Auf Grund von eigenen Beobachtungen kann empfohlen werden, dabei auch die äussere Betrachtung der Agglutinationsform nach dem Zentrifugieren als Hilfsmittel zum Schutz vor Fehlerquellen zu benutzen: bei den homozygoten Typen schwer trennbare fetzige Agglutinate, beim heterozygoten Typ leichte Trennbarkeit durch Schütteln.

Für die Annahme einer besonderen O-Substanz dürfte das bisher vorliegende Material nicht hinreichend erscheinen. Man muss die verschiedene Affinität der einzelnen Partialantikörper im normalen Blutserum in Rechnung ziehen. Die beschriebenen Tatsachen, insbesondere die α_2-Wirkung lassen sich dann auch im Sinne einer schwach aviden artspezifischen Antikörperwirkung erklären.

*Dr. F. **Schiff** (Berlin): *Abbau gruppenspezifischer und verwandter Substanzen durch Fermente und Bakterien.*

Die von O. Sievers gefundene Verstärkung des "Blutgruppenfermentes" durch Pepton braucht nicht als Nährbodenwirkung im Sinne von Sievers und damit als Hinweis auf ein belebtes Agens aufgefasst zu werden, es könnte sich vielmehr auch um Aktivierung eines gewöhnlichen Fermentes handeln. Zur Entscheidung der Frage wurden Versuche unter *sterilen* Verhältnissen ausgeführt (Fermentlösung: Berkefeld-Filtrate aus autolysiertem Speichel). Pepton verstärkte auch unter diesen Bedingungen die Abbauwirkung beträchtlich, sodass es sich hier jedenfalls nicht um eine Wachstumsförderung aerober oder anaerober *Bakterien* handeln konnte. Da in dem Material auch ein Passagevirus in Sinne von Witebsky-Satoh nicht nachweisbar war, so sprechen die Versuche für die Annahme einer Aktivierung bzw. Verstärkung eines im Speichel enthaltenen unbelebten Fermentes. Eine entsprechende Verstärkung des "Blutgruppenfermentes" aus Speichel üben auch an sich unwirksame Kochextrakte aus *Faeces* des Menschen aus; hierdurch erklärt sich zumindest teilweise, dass frische Faeces Gruppenstoffe weit kräftiger abbauen, als Speichel dies vermag. Gegenüber der Abbauwirkung des Gasbazillus Fränkel-Welch wurde ein Unterschied im Wirkungsbereich festgestellt: Speichel und Faeces greifen *alkoholische* Extrakte aus A-Erythrozyten und A-Faeces nicht an, dagegen kommt dem Gasbazillus F.-W. diese Fähigkeit zu. Weiter wurde gefunden, dass auch das *Forssmansche Antigen* aus Meerschweinchenniere, welches durch gruppenabbauende Stuhlextrakte des Menschen nicht angegriffen wird (vgl. M. Eisler) und über dessen Abbau durch Fermente oder Bakterien

bisher positive Befunde nicht vorliegen dürften, unter den Einfluss des Bac.F.-W. serologisch unwirksam wird.

Dr. D. Matta (Cairo): *Study of the Distribution of Group Specific Substances.*

I. *Subgroups.*

(1) A_1 and A_2 were differentiated by the aid of natural α_1 and α_2 agglutinins and by titration and absorption methods.

(2) B_1 and B_2 were differentiated by β_1 and β_2 agglutinins discovered in group B sera and by titration and absorption methods.

(3) The subgroups obtained were: A_1, A_2, B_1, B_2, A_1B_1, A_1B_2, A_2B_1 and A_2B_2.

(4) α_1 and β_1 were of the same nature as α and β agglutinins, although weaker through absorption with A_2 and B_2 cells, therefore agglutinated the sensitive A_1 and B_1 cells. A_2 and B_2 cells, at low temperature, absorbed α_1 and β_1 respectively.

(5) α_2 and β_2 agglutinins were specific for O cells, and agglutinated A_2 and B cells, since they contained O substance. Accordingly A_1 and B_1 differ from A_2 and B_2 both qualitatively and quantitatively.

II. *Preparation of Immune Anti-O.*

(1) Both goats and rabbits were immunized with O and A_2 cells. Sera obtained were absorbed with A_1B_1 cells, the serum of which contained anti-O.

(2) According to the strength of reaction with the absorbed serum, the following types were found :—

Reaction	Titre.	
Very strong	(1–128)	(1) all O cells.
Strong	(1–64)	(2) all A_2, (3) all B_2 cells.
Moderate	(1–16)	(4) some A_1, (5) some B_1, (6) all A_2B_2 cells.
Weak	(1–4)	(7) some A_1, (8) some B_1, (9) all A_1B_2, (10) all A_2B_1 cells.
Negative	—	(11) some A_1, (12) some B_1, (13) all A_1B_1 cells.

The same reactions were obtained from goats and rabbits, both immunized with O or A_2 cells.

This serum is different from Anti-M and N and specific to a substance found in most bloodtypes. It is best developed in O cells and may be called O substance, irrespective of its nature. The strong reactions with A_2 and B_2 cells constitutes another proof that they contain O substance. The four types found in groups A and B may be differentiated by the decrease in strength of the reaction with Anti-O and the increase in strength of the reaction with Anti-A and B respectively. The presence of O substance in A_2B_2 cells is unexpected according to Bernstein's theory.

III. *The inheritance of O substance.*

The examination of bloods of 97 families comprising 400 persons with their serum showed that O children are only found

when the bloods of both parents reacted moderately or strongly with Anti O.

Accordingly A_2B_2 parent may have an O child, provided the other parent reacts at least moderately.

In this may lie an explanation of the Haselhorst exception and of the unexpected high percentage of illegitimate children found when one or both parents are of group AB.

Dr. E. Witebsky (New York): *The General Aspect of the Problem of Organ Specificity.*

Injection of saline suspensions of organs into rabbits may lead to the formation of the following antibodies: (*a*) species specific, (*b*) heterogenetic, (*c*) group specific, (*d*) organ specific. Antisera may contain simultaneously either one or several of the different antibody types. Organ specific antigens may be present:

(1) In all homologous organs of different species,

(2) In many but not in all homologous organs of different species,

(3) In one organ of one species only, and finally

(4) Specific cell properties may be found in organs which are altered by disease.

According to our present knowledge only organs of the nervous system and its derivatives belong to the first group, with the exception of the crystalline lens. Serological methods have proven the presence of common antigens as well as of special ones found only in certain portions of the nervous system. Alcohol soluble substances are mainly responsible for this type of organ specific qualities. The serological differences between fœtal and adult brain on the one hand and between fish and mammalian brain on the other raise ontogenetic as well as phylogenetic problems.

Thyreoglobulin is the representative of the second group of organ specific antigens.

As an example of the third group the red blood cells of the guinea-pig and of the horse may be mentioned. They contain organ specific antigens which are limited to the respective species.

Within the fourth category specific antigens of malignant tumours are of special interest. Saline suspension of malignant tumours sometimes produces tumour specific antisera when injected into rabbits. Such specific antisera may react *in vitro* with alcoholic extract of malignant tumours which by themselves do not induce the formation of tumour specific antibodies. The question of tumour specific antigens needs, however, further investigation.

Prof. R. Bruynoghe (Louvain): *Les agglutinogènes des globules humains.*

Les agglutinogènes A et B sont thermostables au point de vue de l'aptitude de la fixation des isoagglutinines.

Pour ce qui concerne leur pouvoir antigénique, seul l'agglutinogène A résiste au chauffage, ce qui est vraisemblablement à mettre sur le compte de la présence de l'antigène de Forssman dans cet agglutinogène.

Les agglutinogènes A et B sont protides, les agglutinogènes supplémentaires M et N des lipides.

Pour autant qu'on peut juger de leur constitution par le comportement de leurs isoagglutinines et de leurs immun-agglutinines vis à vis des substances étrangères, les agglutinogènes A et B sont de structure complexe.

En dehors de leur antigène spécifiquement humain, ils renferment des substances identiques à certains constituants des globules ou des tissus d'animaux.

L'antigène hétérogénique présent dans l'agglutinogene A n'est pas tout à fait identique à l'antigène de Forssman d'autre provenance.

Les extraits d'organes d'animaux et entre autres de lapins peuvent des fois contenir des substances susceptibles de fixer les agglutinines anti-M et anti-N. Cette particularité fournit l'explication de ce que généralement nombre de lapins fournissent des sérums anti-M et anti-N inactifs, malgré qu'ils ont subi de nombreuses injections de globules pourvus de l'un ou l'autre agglutinogène supplémentaire.

Dr. F. Ottensooser (Bern): *Unterscheidung menschlicher Blutkörperchen mit polyvalentem Immunserum.*

Zur Unterscheidung menschlicher Blutkörperchen sind polyvalente Hetero-Immunsera besser geeignet als tierische Normalsera.

Die Immunsera wurden in folgender Weise hergestellt: Von 9 Kaninchen erhielt jedes eine bunte Mischung menschlicher Blutkörperchen, die aus Blut aller Gruppen und Typen oder aus O-Blut der verschiedenen Typen bestand. Bei zwei von den Immunsera war die individuelle Spezifität besonders ausgeprägt, sie wurden gemischt und mit den zu prüfenden Blutkörperchen abgesättigt. Dabei liessen sich *alle untersuchten Blutproben*, z.B. auch die mehrerer OMN-Personen, *auseinanderhalten*; die Blutkörperchen irgend eines Menschen entfernten aus dem ausgeglichenen, auf A- oder B-, M- oder N-Blut gleichstark wirkenden Antikörpergemisch elektiv das auf ihr Antigenmosaik passende Antikörpermosaik.

Doch ist die individuelle Spezifität bei den Hetero-Immunsera geringer als bei den früher beschriebenen Iso-Immunsera gegen Tierblut.

Die Hetero-Immunsera gegen Menschenblutkörperchen konnten nur unvollständig abgesättigt werden, sodass der Abguss zwar fremdes Blut stärker agglutinierte als das zugehörige, aber auch mit diesem noch eine, wiewohl schwache Reaktion gab. Häufig reichte die individuelle Spezifität für eine doppelte Absättigung nicht aus

sondern erlosch, wenn der Abguss von den Blutkörperchen einer Person noch mit denen einer zweiten abgesättigt wurde. Daher ist es auch nicht regelmässig gelungen, mit den Blutkörperchen der Eltern die Antikörper gegen die Blutkörperchen des Kindes elektiv zu entfernen und es steht nicht fest, dass es erbliche Merkmale sind, die das Hetero-Immunserum über die der bekannten Gruppen und Typen hinaus erfasst.

Dr. P. A. Gorer (London): *Antigenic Differences in Pure Lines of Mice.*

The term " pure line " is here used to signify a line that has been brother-sister inbred for over twenty generations. Three such lines have been used: "Agoutis," "Albinos " and " Blacks."

Most of the Agoutis reacted very weakly with human group A serum, with the exception of some males, which gave relatively strong reactions, especially at room temperature. When absorption tests were performed at this temperature, it appeared as though the line were antigenically heterogeneous. Breeding tests failed to demonstrate any genetic impurity in this strain and absorption tests at 37° C. showed no evidence of antigenic differences. It was concluded that sera of this type give non-specific reactions at room temperature (about 20° C.).

Using sera from immunized rabbits it has been possible to detect the following antigens in the three lines:—Agoutis No. I; Albinos, Nos. I and II; Blacks, No. IV.

No evidence has been found to indicate the presence of genetic or antigenic differences *within* the lines. However some of the albino males give very weak reactions with antibody II. If Albino x Black, F_1 hybrids are backcrossed to the Albinos, one-half of the backcross generation possess antigen IV. In this case it was found that the type of reaction with antibody IV varied in exactly the same way as did the pure line with antibody II. No such variability has been observed in the pure line of Blacks with any sera.

It is concluded that differences in agglutinability between different erythrocytes are not *necessarily* correlated with antigenic differences (quantitative or qualitative). Variations of the type described in the Albino strain may account for the intermediate types found in the subdivisions of the human group A. Such non-specific variations in sensitivity are only partially controlled genetically.

Dr. D. Harley (London): *Some Possible Sources of Error in Medico-Legal Blood Group Determinations.*

(1) ***The Roche Lynch-Martley technique for determining the A and B agglutinogen content of dried blood stains by the absorption of***

O sera. This method is especially suitable for small stains, approximately 0.5 sq. cm. of stain and 30 to 40 c.mm. of serum being employed. The value of this technique depends on attention to the following: (*a*) The α and β agglutinin titres of the absorbed serum should be compared with those of unabsorbed serum and of serum absorbed with unbloody material. The reason of this is that many cloths and other materials may absorb varying amounts of agglutinin. (*b*) As an A_2 corpuscle suspension is more suitable than an A_1 for the test after absorption with a group A_2 stain, tests are made with A_2 corpuscles, or preferably with both A_1 and A_2. (*c*) Certain O sera show a marked loss of β agglutinin after absorption with A corpuscles; therefore I employ only those O sera which show no reduction of heterologous agglutinin when absorbed with known A and B corpuscles.

(2) *A false agglutination reaction with anti-M and anti-N sera.* An unusual type of reaction was observed with anti-M serum and corpuscles lacking M agglutinogen (and with N serum and corpuscles lacking N) when put up on glass slides and gently rocked; the corpuscles often agglutinated into definite clumps, but when allowed to remain at rest, these clumps disintegrated and the corpuscles became evenly distributed. This phenomenon was found with sera of 1 : 10 dilution which had not been further diluted for the tests. On diluting to about 1 : 30 this does not occur. Individual sera vary, but it was found more with a brand of commercial serum than with the writer's own preparations. I suggest this may be due either to some non-specific physical property of rabbit serum or to traces of species agglutinins from incomplete absorption. The importance of this effect on the practice of M and N typing by the slide method is obvious. Each corpuscle suspension should be put up with a series of dilutions of the test sera, and doubtful reactions examined after the slide has been at rest for ten minutes. Lastly, commercial sera should only be employed after testing on known M and N bloods, and best only as an independent check on the investigator's own preparations.

Dr. S. C. Dyke (Wolverhampton): *Practical Considerations governing the Determination of Compatibility for Blood Transfusion.*

In the main the original findings of Landsteiner must still govern the precautions to be taken in determining compatibility. Symptoms after transfusion due to "incompatibility" occur only after infusion of red cells susceptible of agglutination by serum of recipient. For purposes of transfusion knowledge of groups of donor and recipient is not essential; all that is necessary is knowledge that serum of recipient will not agglutinate red cells of donor. In the absence of this knowledge, and if reliance is

placed on compatibility of groups alone, trouble may arise from presence of unsuspected agglutinins in serum of recipient. Evidence exists of occasional presence in serum of isoagglutinins other than anti-A and anti-B and capable of acting upon red cells O. These may be anti-A2 agglutinins. In repeated transfusions trouble may be experienced from agglutinins called into existence by previous infusions. These may be against factors M and N. The presence of such unexpected agglutinins, if capable of acting upon the red cells of the proposed donor, will be unmasked by preliminary direct matching.

The simplest technique is the best, for grouping serum must be of high agglutinin titre; pooled serum may be raised to the requisite high titre by concentration.

"Cold" agglutinins occur more commonly in normal serum than is usually supposed, and frequently in that of anæmic subjects. Their misleading effects may be guarded against by performing matching tests in the warm incubator. In the case of proposed recipients showing cold agglutinins in the serum, the infusion of blood of the same group may be performed with impunity, provided that it be kept warm.

For the purposes of transfusion, a compatible blood is one the red cells of which are not susceptible, except at low temperature, to agglutination by the serum of the proposed recipient. The infusion of blood satisfying this criterion will never give rise to "transfusion shock."

Dr. V. Friedenreich (Copenhagen): *A hitherto undescribed Type of Blood Corpuscles A (A 3).*

This paper concerns a very rare type of A-cells, which, as it most probably represents a new serological sub-group (genetically: group) within the original O-A-B-System of Landsteiner, is designated A_3.

Serology.—(1) Main characteristic: far weaker than A_2, i.e., weaker than or equal to the A_2-agglutinogen in A_2 B-cells, and, so, only demonstrable with specially strong sera. (Preparations illustrating this were demonstrated: *a*). An anti-A-serum giving almost maximal reaction with A_2-cells, no reaction with A_2B or A_3; *b*) 3 chance commercial test-sera giving practically or positively no reaction with A_3).

(2) *Absorption-power:* between that of A_2 and A_2 B.

(3) *Reaction with aglutinin α_1:* almost like A_2.

(7) *Serum:* content of anti-A-agglutinin never observed, and only in some few instances cold-agglutinin α_1 was found.

(5) *Variability:* very slight; no transitions to A_2 observed.

Frequency: as yet unknown (personal experience: six examples, independent of one another, in ca. 5,000 blood samples).

Family examinations: six families (260 members), including 76 A_3- and 3 A_3 B-individuals. Pedigrees were demonstrated. Chief deductions thereof:

(1) No A_1- or A_2- offspring from $A_3 \times O$ matings.
(2) A_3- offspring is seen from A_1- or A_2- parent.
(3) A_1- parent with A_3- offspring has no O- children.
(4) No A_3- offspring from matings between a non-A- individual of the family and a foreign A- mate. Some segregation numbers were shown.

The facts can hardly be explained in any other way than by the supposition of a 3' allelomorphic gene, i.e., a gene A_3, which is dominated by A_1 and A_2 like A_2 by A_1.

Some consequences of this system are mentioned. One single exception from the rule (an O- child from a parent (father!) of the genotype A_2, A_3) was discussed.

The practical importance of the "new" group (possibility of erroneous blood-grouping) was briefly discussed.

Prof. O. Streng (Helsinki): *Die Blutgruppenforschung und die Anthropologie.*

Schon im Jahre 1926 habe ich gezeigt, dass man die Blutgruppenresultate bei den Völkern mathematisch genau in ein gleichseitiges Dreieck einzeichnen und graphisch richtig wiedergeben kann.

Später habe ich gezeigt, dass die Völker, genügend grosses Material vorausgesetzt, einen bestimmten Platz in der Blutgruppen- Punktkarte haben, welcher Platz mathematisch genau die serologische Zusammensetzung eines Volkes angibt, welches auch aus der hier vorgelegten Karte zu sehen ist.

Es wäre wünschenswert die Resultate von der Punktkarte mathematisch richtig in die geographische Karte überführen zu können, wo man die serologischen Ähnlichkeiten und Unähnlichkeiten mit der tatsächlichen Nachbarschaft und Umgebung direkt vergleichen und überblicken könnte.

Um die Resultate geographisch überblicken zu können hat *Furuhata* 1933 die verschiedenen Gebiete Japans sowohl in der Punktkarte als in der geographischen Karte mit Farben bezeichnet. Die Farben sind aber willkürlich gewählt und die Methode bedeutet m.E. keinen methodischen Fortschritt.

Wenn *jeder* Punkt in dem Dreieck seine verschiedene Farbe und gleichzeitig die nebeneinander liegenden Völkerpunkte ähnlichartige und die fernerliegenden mehr abweichende Farbennüancen hätten, und diese Farben in die geographische Karte eingeführt würden, hätte jedes einzelne Volk und jedes Land nicht nur seinen eigenen bestimmten Platz in der Punktkarte, sondern auch in der geographischen Karte seine eigene, von diesem Platz abhängige und von der serologischen Zusammensetzung des Volkes bedingte Farbe.

Mein Assistent, Dr. *K.O.Streng* hat dieses Problem sich vorgelegt. Er hat die Spektralfarben des Mantels des *Ostwaldschen* Farbenkegels benutzt. Man hat sich die Oberflächenfarben des Ostwaldschen Doppelkegels in eine Ebene ausgebreitet und in die Punktkarte hineinzudenken. Die Wandlung schwarz in weiss folgt der Richtung der r-Achse des Dreiecks, die Spektralfarben folgen der q-Achse oder einer in der Mitte zwischen p und q gelegenen Achse.

Schon beim ersten Blick auf die verschiedenen Europa- und Weltkarten sieht man, dass die Farben ganz wie in der Punktkarte bestimmten Regeln folgen, so dass die serologischen Ähnlichkeiten der Völker und ebenso die Übergänge zu den Nachbarvölkern sehr übersichtlich zu sehen sind.

Alle Völker sind noch nicht in genügendem Umfange untersucht worden um eine *endgültig richtige* Punkt- und Farben-Weltkarte über die serologische Zusammensetzung der Menschheit zu ermöglichen.

Um die Bedeutung der Blutgruppen endgültig feststellen zu können, sollte man serologische und anthropologische Bestimmungen in grosser Ausdehnung gleichzeitig machen müssen. Analoge anthropologische Punkt- oder Farbenkarten z.B. über das Vorkommen der Doliko- und Brachykephali u.s.w. unter den Völkern wären wünschenswert.

Ein brauchbares Material wird gesammelt, wenn die Wehrpflichtigen der verschiedenen Länder serologisch und gleichzeitig anthropologisch im *grossen Umfange* untersucht werden. Ich möchte den Vorschlag machen, dass man auf diesem Wege in den verschiedenen Ländern vorwärts gehen möchte und dass die serologischen Feststellungen dabei von geübten Serologen gemacht würden.

Dr. P. Levine (Newark, N.J.).

It was my belief that the rules for finding compatible donors, namely, selection of individuals within the same group, followed by a direct test of patient's serum and donor's cells, were firmly established. I am, consequently, very much surprised to hear Dr. Dyke's remarks that "knowledge of groups of donor and recipient is not essential." In resorting to direct tests only, if no agglutination occurs in mixtures of patient's serum and donor's cells, then either (1) both belong to the same group, or (2) donor is in group O, or (3) there may be the rare combination of weak agglutinins and poorly sensitive cells. The latter type of incompatibility can be detected only by a grouping of the red cells. Granting that such a transfusion may possibly prove uneventful so far as immediate symptoms are concerned, certainly the donor's blood cells will be rapidly eliminated from the circulation.

As to group O donors, the concensus of opinion is against the indiscriminate use of universal donors. Another serious objection

to the exclusive use of direct matching is that, in certain cases, it may become an unnecessarily tedious process, for it is obviously simpler and less time-consuming to detect incompatibility by grouping of the cells.

It is my opinion that the observations of Matta and Thomsen are compatible with the Bernstein theory. The exceptional cases of AB parents having an O child may be explained by chromosomal non-disjunction (Levine, Haldane).

I am glad to announce that legislation authorizing American courts to order blood tests in paternity disputes has already begun. Such laws are now on the statute books of New York and Wisconsin.

Dr. E. Berger (Basel).

Als Beitrag zu den Ausführungen von Herrn Dyke und Herrn Witebsky möchte ich über Untersuchungen berichten, die Herr Dr. Fritz Köpplin in Basel auf der Medizinischen Universitätsklinik des Herrn Prof. Staehelin ausgeführt hat.

Herr Witebsky führte in seinem Vortrag als eine 4. Form der Organspezifität antigene Eigenschaften an, die bei pathologischen Veränderungen auftreten. Hierhin dürften die von Dr. Köpplin gemachten Beobachtungen gehören, in denen zunächst in einem Fall von Perniziöser Anaemie eine *Autohaemagglutination* nachgewiesen wurde. Im Immunisierungsversuch mit den gewaschenen Blutkörperchen des betr. Patienten wurden von Kaninchen Antisera gewonnen, die nach Absättigung mit den Erythrocyten der verschiedenen Blutgruppen frei von Hetero- wie gruppenspezifischen Agglutininen waren und nunmehr nur noch die Erythrocyten des Patienten agglutinierten; dies aber, wie zu erwarten, nicht nur bei niedriger, sondern auch bei höherer Temperatur. Der gleiche Befund konnte einige Zeit darauf bei einer Patientin erhoben werden, die an paroxysmaler Haemoglobinurie und haemolytischem Ikterus erkrankt war. Auch die Erythrocyten dieser Patientin zeigten das Phaenomen der Autohaemagglutination und lieferten, Kaninchen injiziert, Antisera mit den beschriebenen Eigenschaften. Ausserdem ergab sich, als die mit den Blutkörperchen der beiden Patienten gewonnenen Antisera kreuzweise mit den Erythrocyten der beiden Patienten im Agglutinationsversuch geprüft wurden, dass die Agglutinine sich serologisch identisch verhielten, ein Befund, der mit Wahrscheinlichkeit dafür spricht, dass in beiden Fällen dasselbe Sonderantigen in den Blutkörperchen vorhanden war.

Prof. H. Sachs (Heidelberg): *Zur Frage des besonderen O-Receptors.*

Für die Frage, ob eine besondere O-Substanz anzunehmen ist, hat die heutige Diskussion, wie ich glauben möchte, keine positiven Anhaltspunkte gebracht. Das gesamte Tatsachenmaterial scheint

mir in der Tat mit der Annahme vereinbar, dass es sich bei der α2 Wirkung um eine artspezifische Antikörperfunktion handelt. In AB-Blutproben, die α2 enthalten, könnten demnach sehr schwach avide *artspezifische Antikörper (α2) schwach aviden artspezifischen* Rezeptoren gegenüberstehen, ohne miteinander in Reaktion zu treten. α2 wirkt wegen seiner schwachen Affinität nur auf O- und α2-Blutkörperchen, die eben durch besonders starke Avidität der artspezifischen Rezeptoren gekennzeichnet sind. Ich glaube, dass man zu einem befriedigenden Überblick gelangt, wenn man die Zusammensetzung der Antigene, aber auch der Antikörper aus *zahlreichen* durch ihre *Affinität* unterschiedenen *Gliedern* in Betracht zieht. Die Erscheinungen, die im Sinne von besonderen O-Charakteren gewertet werden, können dann der Ausdruck artspezifischer Antikörperwirkung sein. α2 wäre dann ein artspezifischer Antikörper von schwacher Avidität, während α1 als ein schwach avides Anti-A aufzufassen ist.

Dr. Rina Younovitch (Tel-Aviv, Palestine).

The mixed population of Palestine has been tested for blood groups; over 10,000 tests on Jews according to their country of origin show figures with Hirszfeld Index of over 2·0 for the German Jews, and under 1·0 for the Buccharian Jews.

The Samaritans, a closed community with intermarriages were tested both serologically and anthropologically. The close relationship of the members helped the study of the law of heredity in the blood groups.

Tests for blood transfusions were always confirmed by serological cross-examinations, both of the donor and recipient.

Special attention was given to patients with anaemia and icterus, when no universal donor can be used. The most careful choice of the donor ought to be made for small children (under a year) having no agglutinins.

Blood transfusion of a small amount of blood of different incompatible blood groups has been done with the special purpose of provoking the reaction.

Dr. A. v. Jeney (Debrecen, Hungary).

The error in the determination of the blood-groups, as it is generally made with only one pair of test-sera, amounts to 5–10 per cent. approximately. If one of the test-sera used has a low titre of agglutinin (α or β) we get, instead of AB group, more cases of A or B group, and instead of A or B group, more cases belonging to the O group. In order to avoid this source of error, it is necessary to work with test-sera of high agglutinin titre, and there are cases in which we have to use two or three pairs of test-sera with high agglutinating power, because not every α-serum agglutinates equally every A-blood cell, and not every β-serum agglutinates every B-blood cell.

On the other hand, the exceptionally high agglutinin-content of blood can be a source of danger in those cases in which an O-type blood, as the blood of a so-called " universal donor " is transfused to a man with a different type of blood. In these cases the agglutinin content of the donor's blood will not be sufficiently diluted and therefore reacts with the blood-cells of the recipient. It is better to give up the whole conception of the " universal donor " altogether, and to use in every case blood-cells from the same type. The safest procedure is to make cross-agglutination in every case with the blood of the donor and that of the recipient.

Dr. S. Marbais (Livry-Gargan, France) : *Spécificité glandulaire. Diagnostic de sexe du fœtus. Diagnostic des maladies microbiennes par la mesure de l'excitabilité.*

Lorsqu'on inocule du testicule à un lapin mâle, la pression artérielle baisse, lorsqu'on l'inocule à une femelle, la pression ne baisse pas. Il y a donc un choc chez le mâle uniquement. Ce choc n'a plus lieu si on inocule le testicule à un mâle décérébré. Cela nous prouve que la sécrétion interne du testicule existe dans le cerveau du mâle. En opérant de même sur un lapin châtré, la pression ne baisse plus à partir du 13e jour de la castration. Le même phénomène existe avec l'ovaire et la rate.

En inoculant du testicule à une femme enceinte si la pression radiale baisse, la femme a un garçon. Elle a une fille si la pression ne change pas ou si elle augmente.

Ces recherches de physiologie m'ont permis de poser le diagnostic microbien des maladies expérimentales et humaines, recherches que j'ai condensées dans un volume spécial[1] résumé dans la séance d'hier.

Mr. P. Bruce White (London) : *The Origin of the Iso-agglutinins.*

The theory of the genetic determination of the iso-agglutinins of normal blood has the stamp of improbability. Some continuous antigenic stimulus is to be suspected. The possibility that this stimulus may be furnished by the material eliminated from the corpuscles during their maturation seems worthy of consideration. The observations of Ito and others suggest that the nucleated corpuscles of the embryo, even in families lacking both A and B, are AB. If the hæmopoietic cells should retain AB while the other fixed tissues were, by genetic determination, O, A, B or AB, then in three of the four cases the corpuscles must threaten, unless some levelling process occurs, to be antigenically superior to the body and to incur the results of an antibody reaction. I suggest that some such incompatibility of corpuscles and tissues is threatened and

[1] S. Marbais. Théorie cérébrale de l'immunité et de l'anaphylaxie. N. Maloine. Edit. Paris, 1934.

that at maturation the offending antigen (AB, B or A as the case may be) is eliminated from the corpuscles and that against these eliminated antigens the isoagglutinins arise. It is to be recalled that in that condition, pernicious anæmia, in which immature corpuscles circulate, autoagglutinability and panagglutinability of the blood are notorious.

DISCUSSION *on* THE SIGNIFICANCE OF ALLERGY IN DISEASE

Chairman : Prof. W. W. C. Topley (London).

Prof. E. L. Opie (New York), **Opener.**

It is desirable as originally proposed to apply " allergy " to the changed state in relation to antigens following their introduction into the body, rather than to sensitization alone. It includes the tissue changes at the site of contact with antigen and the acute functional disturbances, such as anaphylactic shock, following dissemination of antigen by way of the blood stream.

The prevailing conception of tissue sensitization would be clarified if it were widely recognized that it is the ability to react with rapid and intense inflammation to an irritant that ordinarily produces scant inflammatory reaction. The term " allergic inflammation " is applicable to the local reaction of sensitization.

Both anaphylactic shock and allergic inflammation produced by foreign proteins are antigen-antibody reactions, subject to passive transfer and to desensitization by excess of antigen. It is noteworthy that both can be passively produced by reversing the order of introducing antigen and antibody, that is, by passively preparing with antigen and later introducing antibody. This reversed reaction has served " to remove some of the mystery from anaphylaxis," and the same statement is applicable to " allergic inflammation."

In the normal animal foreign protein, for example, crystalline egg albumen, introduced into the skin of a rabbit, spreads widely but produces scant inflammation and readily enters the circulating blood. In the sensitized animal it produces intense inflammation (Arthus phenomenon), remains localized and fails to enter the blood. Both antibodies and inflammation favour the local fixation of the antigen.

The tuberculin-like reactions have characters of their own, for they are elicited in sensitized animals by substances, e.g., tuberculin, that do not have the characters of an antigen and cannot produce sensitization or immunity. The relation of " atopens " to antigens is as yet ill defined and the great domain of atopic sensitization requires further exploration.

It has long been known that immunity characterized by antibody formation and heightened resistance may occur in the absence

of sensitization. Allergic inflammation under some conditions may be an unessential aspect of protection against antigenic agents. Nevertheless, a review of the changes following sensitization to the tubercle bacillus or to streptococci or their products shows that it modifies profoundly the pathogenesis of the disease.

Tubercles are formed more rapidly in the allergic than in the normal animal and recent observations have shown that both polymorphonuclear leucocytes and macrophages accumulate more rapidly in the former. In the lungs, liver, spleen and other organs of allergic animals that resist infection, tubercle bacilli are destroyed more rapidly than in animals that have been normal before inoculation. Sensitization may favour the occurrence of caseation, but tuberculous lesions are more extensive and caseation less conspicuous in allergic than in previously normal animals, provided the microorganism is not present in such number that it multiplies without restraint. The resolution of tuberculous pneumonia occurs more readily in the allergic animal.

There is widespread inclination to refer the unexplained characters of certain diseases to allergy and some trustworthy observations have been assembled in support of this view. Diseases that have attracted most attention in this relation are lobar pneumonia, rheumatic fever, glomerular nephritis, eclampsia, etc. Exact criteria are essential; the presence of an antigen capable of producing, under the conditions of their occurrence, the symptoms and lesions of the disease, must be demonstrated, but this information is, in large part, lacking. Nevertheless, further knowledge of the pathogenesis of infectious diseases is conspicuously dependent upon a better understanding of the influence of developing sensitization and resistance upon their functional and anatomical characters.

Prof. F. v. Gröer (Lwów): *The Principles of Allergometry.*

Allergometry is a method which permits detection and measurement of the actual and individual ability to respond to the irritating action of a given allergene.

The method is based upon the following general biological law discovered and formulated by v. Gröer and Steinhaus.

The maximal degree of response to the irritating action of the stimulus stands (during a conveniently short period of observation to avoid a noticeable change in the behaviour of the living substance) in the same relation to the negative logarithm of the concentration of the stimulant, as does the ordinate to the abscissa of a generalized hyperbola.

The generalized hyperbola ($x^a y^b = k$), and only the generalized hyperbola, possesses the most important property of yielding a straight line, if submitted to logarithmic anamorphosis.

If, therefore, the individual tested is subjected within a conveniently short period of time to repeated stimulation by means of various concentrations of the same allergene (for practical

purposes only two such observations are necessary), but remains otherwise under constant conditions, and the resulting responses are measured and their maximal size plotted against the negative logarithms of the test concentrations of the allergene on a *xy*-plane, *with both axes logarithmically scaled*, then the result will be a straight line. This straight line expresses the actual, individual state of allergy of the tested individual towards the allergene used. Each straight line can be determined by means of two parameters. For instance, by means of the sectors, which the straight line, thus obtained, cuts off both the axes *x* and *y*. These two parameters are called: S or *Susceptibility* of the tested individual to the damaging action of the allergene and R or *Reactivity*, the faculty of the tested individual to respond to this action of the stimulus.

S and R vary not only from individual to individual, but also in the same individual *with time*.

On an RS-plane, with S as abscissa and R as ordinate, the existing individual state of allergy appears as a *point*. The position of such a point renders the actual picture of the individual state of allergy of the examined individual. There are three fundamental possibilities, as regards the position of such a point in the RS-plane and thus of the actual state of allergy: The homodynamic (R = S), the heterodynamic-pleoæsthetic (R<S) and the heterodynamic-pleoergic (R>S) state of allergy. As the parameters S and R change with time the position of the individual point varies accordingly and describes a curve. This curve may be called the " path of allergy."

Determination of the actual individual state of allergy and tracing the " path of allergy " have proved of the greatest value for the study of allergic diseases.

(The details of the methods of allergometry were shown by means of a cinematographic demonstration.)

Prof. H. Zinsser (Boston, U.S.A.): *Principles of Hypersensitiveness.*

It is desirable to redefine some basic conceptions in terms of direct observation. Hypersensitiveness exists when an individual reacts to a foreign substance differently, in intensity or kind, from normal individuals of the same species. Its expression differs with the type of animal; too ready generalization has led to error. The hypersensitive state depends on previous contact with an antigenically identical or related substance. Phenomena seemingly indicating hereditary or genetic hypersensitiveness are due to sensitization *in utero* or by alimentary or respiratory routes; families may be affected, but the specificity of hypersensitiveness is not hereditary and symptoms usually develop years after birth; a hereditary capacity for sensitization is alone to be admitted.

Preceding exposure enhances the capacity of the cellular structures to react to the foreign substance; this enhancement constitutes the symptoms of hypersensitiveness.

It had once been believed that true hypersensitiveness developed only to antigens stimulating circulating antibodies but substances individually inert (e.g., bacterial polysaccharides, Forssman lipoids, even formaldehyde, iodine) may unite with proteins—possibly *in vivo*—thereby becoming antigenic and inducing hypersensitiveness—for the display of which no protein combination is required.

Heterologous passive sensitization is often impossible—guinea-pigs may be sensitized to pneumococcus polysaccharides with antipneumococcus rabbit serum, not with the corresponding horse serum; passive local sensitization (e.g., in the P-K reaction) may be obtained where generalized passive sensitization is not.

Man differs relevantly from other animals in that the acute reactions in man do not often, by virtue of their site, entail death; in longevity, diet, occupations, with resulting wider contacts; in a tendency to localized hypersensitiveness (dermal, respiratory, intestinal) determined by the route of sensitization. We may survey human hypersensitiveness as :—

(1) Forms like serum sickness and certain food idiosyncracies, comparable to true anaphylaxis, traceable to specific sensitization, associated with circulating antibodies, even passively transmissible, with symptomatic modification, to animals. (2 Forms of localized hypersensitiveness, of which the immunological nature is indicated by specificity and local sensitizing capacity of the serum: the serum constituents involved are essentially antibodies. (3) More obscure forms which we are apt to call "allergy," never yet passively transferred (e.g. tuberculin allergy). The following characteristics supply clues to its nature: the significant specificity of such reactions as tuberculin allergy indicate that the stimulant must emanate from the organisms themselves; development of reactivity 2–3 weeks after injection of the organisms, living or *dead*, exclude the agency of a metabolic product; typical reactivity is not stimulated by filtered extracts or without some tissue reaction at the site of injection; in animals, once allergic, typical reaction may be produced by bacterial materials not identical with the carbohydrate haptens, probably heat stable proteins of hapten nature.

One must conclude, it seems, that in the allergic state we deal with a condition induced by products, liberated from the organism during its reaction with inflammatory tissue, which are antigenically denatured in attempting their chemical separation. This type of allergy differs from other hypersensitiveness in that, by no known method, has it been possible to convey the sensitive state.

The immunological definition of antigen must be expanded: there are whole antigens which stimulate circulating antibodies, others which sensitize without inducing such antibodies. The distinction is not fundamental; a production of antibody is incidental; there is throughout the same essential reaction with cells which thereby become sensitized; it is the basic property of altering cellular reacting capacity which binds the phenomena together.

Dr. G. Shwartzman (New York): *Phenomenon of Local Skin Reactivity to Bacterial Filtrates.*

The phenomenon of reactivity described is induced by certain bacterial toxic principles. They are antigenic and can be neutralized specifically by homologous antisera in multiple proportions. The distinction between the toxic principles and true exotoxins lies in the mechanism of effect. If active principles are introduced into the blood stream during the time of reactivity, they produce severe damage in reactive tissues. The state of reactivity is not comparable to any condition hitherto described, and apparently presents some functional disturbance in the cell. A large number of non-bacterial substances failed to elicit this state of reactivity.

This phenomenon is sharply differentiated from anaphylactic phenomena by the following features:

Local reactivity: elicitation of reactivity by single injection; incubation period of few hours; prompt disappearance of reactivity.; serum neutralizability of active principles; inability of animal proteins to induce the reactivity; lack of passive transfer; and lack of specific relation between sensitizing and provocative factors.

Whilst the state of reactivity can be elicited exclusively by certain bacterial principles, potent provocative factors may be obtained not only from bacteria but also from combinations of animal proteins with homologous antisera.

It is essential that the provocative injection be given into the blood stream. The preparatory effect, however, may be elicited in one of the following ways:

(1) Preparatory injection of active bacterial principles into the skin, or parenchyma of internal organs (local perivascular preparation) (Shwartzman).

(2) Preparatory injection into the vascular system of an organ (local vascular preparation, i.e., kidney, ear) (Shwartzman).

(3) Preparatory injection into the general circulation (general vascular preparation) (Gratia and Linz, Apitz, and Gerber). A state of reactivity may also be induced by the injection of live cultures; the distribution of lesions depending on selective localization of bacteria (cholera vibrio) (Sanarelli).

Histologically, the lesions of local and general phenomena show venous thrombosis and diffuse vascular damage. Enhanced fragility of capillaries may be disclosed by suction, etc. Thus, the mechanism consists of two distinct phases:

(1) Elicitation of latent vascular reactivity; (2) Production of vascular lesions when certain provocative factors are superimposed at suitable times.

The provocative factors may be derived from bacteria, viruses, and complexes of innocuous antigens with specific antibodies. This mechanism, therefore, approaches human symptomatic hæmorrhagic diathesis, where bacterial factors alone may be implicated in the production of both phases; and also the "anaphylactoid" hæmorrhagic diathesis, where the combined effect of bacterial reactivity with subsequent response to unrelated anaphylactic processes may be assumed.

Dr. L. Dienes (Boston, U.S.A.): *The Place of Bacterial Allergy in the Immunization Process.*

After injection of an appropriate antigen both in animals and in man the first noticeable sign of the specific response is a slight skin sensitiveness. Three days are usually necessary for its development. Anaphylaxis and antibodies in the serum as a rule do not appear before the seventh day. This early skin sensitiveness represents the development of active immunity in the tissues, and it is not produced by circulating antibodies. The early skin reactions are similar to slight tuberculin reactions; they are delayed in development and persist for several days. In passively sensitized animals the skin reactions appear always in the form of a quickly developing and transient edematous wheal; dilation of blood vessels and edema start immediately and are followed by infiltration with polymorphonuclear leucocytes. The slight early reactions start with perivascular and perineural infiltration in which large mononuclear cells predominate.

If tuberculous guinea-pigs are sensitized with egg white under appropriate conditions, at the end of the first week the slight early reactions are replaced by strong necrotic skin reactions corresponding in every respect to strong tuberculin reactions. These strong necrotic reactions are independent from circulating antibodies. Slight skin reactions in these guinea-pigs, obtained with very small doses of egg white, and slight tuberculin reactions, correspond in their development, gross appearance, and microscopic structure to the slight early skin reactions of actively sensitized animals. It is evident that all these skin reactions indicate varying intensities of the same type of hypersensitiveness.

The conception developed here gives several viewpoints for the understanding of immunity process during the infectious diseases. First, it indicates that bacterial allergy is a sign of the development of active immunity process in the tissues; second, that the

immunological effects of active and passive immunization are of an essentially different nature; third, that the immunity response, when it begins, produces in the tissues exposed to the antigen a cellular reaction consisting mainly of large mononuclear cells. In diseases in which this phase of the immunity response persists, the defensive reaction is mainly a tissue reaction.

Dr. C. Prausnitz (Ventnor, I.O.W.): *Allergy as a Factor in the Respiratory Disease of Cotton Workers.*

The diagnosis of allergic supersensitiveness can be performed by the skin test on the patient, or the passive serum transference test on normal subjects. The skin test is simpler but not quite without risk. The serum test has the further advantage of allowing a large number of comparative and control tests to be carried out. As neither test is always positive, it is necessary to perform both in every doubtful case.

In a recent investigation on the respiratory disease of cardroom workers in the cotton industry (" cardroom asthma "), the cotton dust was found to contain a primarily toxic protein causing intense, persisting leucocytic infiltration after intradermal injection of 1 mgm. to normal men or animals. In doses of 0·001 to ·01 mgm., it invariably produced a typical fleeting skin reaction in all diseased cardroom workers examined, but only exceptionally in the controls. The results were confirmed by the passive serum transference test. One of the controls, in the course of the experiments, became supersensitive to the protein and gave positive skin and serum reactions. This investigation, therefore, provides an example of the more uncommon form of supersensitiveness to a primarily toxic substance.

Dr. J. Freeman (London) said that while the laboratory expert might give, like Professor Opie and von Pirquet himself, a special meaning to the term " Allergy," the general practitioners in England, and also, he understood, in America, implied thereby asthma, hay-fever, urticaria, eczema or something of the sort; certainly they would include hay-fever, as did von Pirquet in his original papers. All these varied superficial symptoms of one underlying serological disease, the toxic ideopathies (Freeman), atopy (Coca), attack diseases (Aschoff) which *most* people called " allergy " did not conform to Professor Opie's definition. An explanation of their marked inheritance (*pace* Prof. Zinsser) was needed: the specific sensitizing process was difficult to demonstrate and in some cases could be excluded. The age-incidence curve of hay-fever rose steeply from birth, formed a plateau between 8 and 15 years and tailed off gradually towards old age; but at no stage of life was the incidence zero, and it was the two ends of the

curve that needed explanation. June babies, it seemed, might be born sneezing; those of April or early May sometimes showed symptoms a few weeks after birth along with the other hay-fever cases in the vicinity. Such babies could not have been sensitized either directly or *in utero* for there had been no grass-pollen. Nor did facts suggest passive sensitization from the mother since all babies of sensitive mothers should then be affected and susceptibility should be transient: actually congenital sensitization was rare but might last half a lifetime.

Food hypersensitiveness presented even greater difficulty. It might be argued that the tissues of a baby reacted violently at weaning to eggs and milk, owing to (hypothetical) sensitization *in utero*, but this explanation failed when strong reaction occurred on eating some exotic food which neither mother nor child had seen before; a case of sensitization to the Chinese *lee-Chee* fruit furnished an example.

Another problem was afforded by obviously hereditary toxic idiopathy appearing late in life: e.g., a man of 70 years stated that his three daughters had all suffered from hay fever, while he—being more intelligent!—escaped; but he developed hay-fever at the age of 75. Why 73 mid-summers failed to sensitize him while the 74th succeeded constituted the problem: something which was not allergic sensitization had happened to this man *in utero* rendering him susceptible in old age. Therefore either hay-fever was *not* allergy, though everyone called it so, or hay-fever *was* allergy though it did not conform to our definition.

It seemed certain that allergic sensitization did not, or might not, take place in the toxic idiopathies.

Dr. E. Witebsky, Dr. E. Neter and Dr. H. Salm (New York): *Production of Hæmorrhagic-necrotic Lesions in the Rabbit by means of Bacteria.*

Three groups of micro-organisms may be differentiated from each other by their capacity to produce hæmorrhagic-necrotic lesions.

When injected into the skin of rabbits, *H. influenzæ* produces inflammation and erythema, but no necrosis in the course of several days. If 24 hours after the intradermal injection an intravenous injection of living or heat-killed *H. influenzæ* is given, hæmorrhagic-necrosis may occur at the site of the intradermal injection. This reaction takes place within three to five hours following the intravenous reinjection.

In contrast, certain strains of pneumococci, when injected intradermally into rabbits cause severe erythema and edema and extensive hæmorrhagic-necrosis may follow (confer Goodner). Blood cultures in this stage are positive. Heat-killed pneumococci, however, are ineffective. When dead pneumococci or *B. typhosum*

agar-washing filtrates prepared according to Shwartzman are injected into the abdominal skin of rabbits and simultaneously living pneumococci into the skin of the back, both sides may show edema and necrosis after 24 to 48 hours. Thus far, it was not possible to reactivate the skin area prepared with dead pneumococci by means of an intravenous injection of living pneumococci, even with massive doses. There is no relation between the type of pneumococci and the reaction mentioned. It is interesting to note that pneumococci are not very suitable for the production of effective exotoxins in the sense of Shwartzman.

There exists a third group of micro-organisms, e.g., Friedlaender's Bacillus, and *B. pyocyaneum*, which again behave in a different way. They may produce hæmorrhagic necrosis when injected into the abdominal skin of the rabbit. Suspensions of dead organisms are ineffective. A skin area injected with heat-killed bacteria, however, may be transformed into a hæmorrhagic-necrotic lesion by means of an intravenous reinjection of the respective organisms.

Dr. **D. Harley** (London): *Bacterial Allergy.*

In past discussions and publications on bacterial allergy and its relationship to anti-bacterial immunity, it does not seem to have been always clearly realized that there are several types of allergic skin reaction to bacteria and their products. The study of tbe skin reactions of rabbits to pneumococci has shown two independent mechanisms giving rise to reactions, one an antigen-antibody union and the other a cellular tissue reaction. There are two pneumococcal allergic reactions of the first or antigen-antibody type, viz., those to the SSS and nucleoprotein fractions. These reactions depend on the presence of circulating type-specific and nucleoprotein antibodies respectively, and the sensitive state is induced by treatment which engenders maximum circulating antibody response, i.e., by intravenous injection of killed vaccine of virulent pneumococci for the SSS allergy, while the nucleoprotein sensitization is produced by virulent and avirulent vaccines alike and is independent of the route of administration. The second, or cellular tissue type, of reaction is exemplified by the reaction of the skin to heat-killed intact organisms. This reaction is a slower one than the first and is essentially inflammatory in character; once established the state remains for long periods, and appears to be independent of both type-specific and nucleoprotein circulating antibodies. This type of allergy is non-type-specific and is induced by treatment with intradermal injections of either virulent or avirulent vaccines. This "intact organism" allergy seems to be of the nature of an increased reactivity or irritability of the skin cells to the nucleoprotein of the intact organism, and follows repeated local reactions of the tissues to the sensitizing injections.

Dr. N. Hamilton Fairley (London): *Allergic Manifestations due to Metazoa.*

Allergic phenomena occur in helminthiasis, infection with warble larvæ, and following the bites and stings of insects and sea-anemones:

Mammalian hosts, especially man, become hypersensitive to the parasites they harbour. The intradermal injection of cyst fluid or a saline filtered extract of larval or adult worm protein has revealed dermal hypersensitivity to many intestinal parasites, including ascariasis (Ransom, Harrison and Couch [1924]) and strongyloidiasis (Fulleborn [1926]), also to hydatid (delayed reaction—Casoni [1905]; immediate reaction—Magath [1921]); schistosomiasis (Fairley and Williams [1927]), trichiniasis (Bachman [1928]), *Filaria bancrofti* (Talliaferro [1930]), *Loa loa* and oncocerciasis (Fairley [1931]). Allergins are demonstrable by Prausnitz-Küstner technique.

Three types of reaction follow intradermal inoculation:

(1) Immediate whealing and erythema;

(2) delayed reaction with extensive puffy swelling of the subcutaneous tissue and hotness and redness of the surrounding skin: occasionally vesiculation occurs;

(3) rarely systemic anaphylaxis with urticaria and asthma.

Conjunctivitis, chemosis, cardiovascular shock, intestinal disturbances, convulsion and coma may follow. Granted hypersensitiveness, dosage is a material factor in determining the onset of (2) and (3). All types of this reaction may occur naturally in man and animals.

Type 1. Immediate whealing following insect and arthropod bites may be of allergic origin; crab-lice, Grübel (1924); flea and midge bites, Boycott (1926); sand-flies, Theodor (1935); "Harara" in Palestine; strongyloid urticaria.

Type 2. Examples include Calabar swelling (*Loa loa*); may follow stable-fly (*Stemoxys calcitrans*) bites and bee and wasp stings,

Type 3. Examples include (1) stings of bees, wasps and medusæ: snake bite; (2) puncture or rupture of hydatid cyst; (3) trichiniasis guinea-worm and *Loa loa*; (4) *Ascaris lumbricoides* and *Parascaris equorum*. Allergic symptoms may follow inoculation of Parascaris fluid into conjunctival sac of infected horses (Weinberg and Julien [1911]). In sheep and cattle harbouring warble larvæ, classical anaphylaxis follows accidental crushing, or injection of warble protein (Hadwen and Bruce [1917]).

Prof. A. Lindau (Lund).

Allow me to call attention to the investigation carried out by Dr. Ahlström of Lund, Sweden. Ahlström has succeeded in localizing the allergic damage in a certain organ of the body—in his studies, the kidneys. We know that staphylococcal toxin has a strong affinity to the kidneys and, given in a sufficient dose, causes

total necrosis of the renal cortex. Into rabbits, sensitized to horse serum, Ahlström injected three hours before the final injection of horse serum, a very small dose of this staphylococcal toxin in a quantity that did not give rise to any anatomical lesions of the kidneys in control animals. This small amount of staphylococcal toxin was, however, sufficient to break down the defence of the kidneys and to localize the allergic attack there. In this way lesions very similar to human glomerulonephritis were produced in the rabbits.

Ahlström's work thus shows us the possibility of localizing the allergic attack in a certain organ, and if in future we succeed in finding substances weakening the defence of the various organs, as specific as the staphylococcal toxin with respect to the kidneys, we may increase our knowledge of several obscure diseases, such as encephalitis, and so on. Ahlström's report is found in this year's issue of Acta Pathologica Scandinavica.

Prof. Th. Madsen (Copenhagen) drew attention to the necessity of using a standardized tuberculin for all comparative epidemiological investigations.

Such standard tuberculin could be obtained from the National Institute for Medical Research at Hampstead and from Copenhagen.

Perhaps the purified tuberculin of American authors (Seibert, Long and others) would be the solution, but further investigation was necessary.

Dr. Marguerite Altoff (Paris) : *Possibilité d'obtenir la réaction hémorragique en préparant l'animal* per os.

Il est possible d'obtenir la réaction hémorragique de Shwartzman en faisant ingérer à l'animal à jeun de l'endotoxine, lysat microbien, à deux ou trois reprises avec 3 jours d'intervalle et en faisant suivre la dernière ingestion par une injection de lysat. (Endotoxine de b. dysentérique des nouveau-nés.) L'animal meurt avec des phénomènes de dyspnée et de convulsions cloniques. A l'autopsie on trouve une hémorragie dans la cavité péritonéale. L'animal témoin injecté avec le même lysat meurt avec des lésions typiques pour la dysenterie, mais sans hémorragie interne.

Prof. M. Heidelberger (New York) seconded Prof. Zinsser's plea for a broadening of the definition of " antigen," suggesting that a simple way would be to omit " circulating " from the portion of the definition dealing with the production of antibodies. He also thought some of the cases Dr. Freeman used might be explained as instances of cross-reactivity, just as *in vitro* reactions might occur between apparently unrelated antigens and antibodies; e.g., gum arabic and Type II antipneumococcus horse serum, Friedländer Type B bacillus and the same serum, etc.

Prof. F. von Gröer (Lwów).

Ich möchte im Anschluss an meinen heutigen Vortrag noch auf die Bedeutung der Pathergometrie für die biologische Auswertung der Toxine hinweisen, worüber wir vorgestern diskutiert haben. Die Differenzen, welche sich bei der Auswertung der bakteriellen Toxine an nicht ganz homogenem Tiermaterial ergeben, und über die besonders Glenny und Prigge berichteten, lassen sich meines Erachtens dadurch erklären, dass die individuelle Pathergielage der zur Prüfung benutzten Tiere nicht immer uniform sein kann. Es gibt eben Tiere, welche eine von dem Durchschnitt abweichende Pathergielage besitzen — wie wir uns inbezug auf das Diphtherietoxin und das Meerschweinchen überzeugen konnten — und solche Tiere müssen dann ganz aus der Reihe fallen.

Prof. von Gröer then continued in English: As a pupil and a co-worker of von Pirquet I feel also obliged to say a few words in answer to the remarks of Dr. Freeman. I think we should keep to the original definition of allergy as it has been given by von Pirquet. Allergy means a different behaviour of the organism towards a stimulant in comparison with the majority of similar organisms. It does not matter whether this different behaviour is due to sensitization or not, whether it is congenital or acquired. It has to be considered by itself as the main feature of this process which we call allergy. A process for allergy is not a static, but a dynamic conception. It changes in the same individual with time. Furthermore, we should not forget that here is also a topographical differentiation of allergy within the allergic individual. The relationship between R and S, according to my scheme of allergy, can vary in the same individual from tissue to tissue. This is the reason why allergy can be produced locally, as we see it in the Shwartzman Phenomenon.

Dr. E. Witebsky (New York).

May I take the liberty to present a working hypothesis concerning the pathogenesis of certain lesions caused by bacteria and their products.

The development of hæmorrhagic-necrotic lesions in the order of experiment of Shwartzman is a double-phased process. The bacterial toxin, injected locally, is first absorbed in the endothelial cells of blood vessels. In analogy to the significance of opsonins in phagocytosis, normal antibody functions of the blood serum are considered important for the intracellular absorption of the locally introduced bacterial substances (the same holds true if the first injection is given intravenously). Thus, antibody functions of the serum are concentrated at the site of the injected area.

If a second injection is given intravenously, the introduced material is conducted to the place where antibodies are accumulated. Morgenroth described a phenomenon called " Ueberspringen von

Amboceptoren." It means that sensitized cells may give up some of their antibodies when non-sensitized cells are added. The reversibility of toxin-antitoxin combinations is also well known. The sudden appearance of new effective substances at the site of the first injection could act corresponding to those observations: A partial dissociation of the antigen-antibody takes place. Thus, toxic substances first absorbed in a neutralized phase may become liberated and exert their toxic action within the cell. The relative lack of specificity of the normal antibody function may explain the fact that a wide variety of substances (but by no means all) may be used for the second intravenous injection.

If this suggestion should lead to further experimental work I shall feel justified in having presented it as a working hypothesis.

SECTION 7

MICROBIOLOGICAL CHEMISTRY

JOINT DISCUSSION *with* Section 6 *on* THE STRUCTURE OF NATURAL AND SYNTHETIC ANTIGENS

Chairman : Dr. P. Hartley (London).

Prof. M. Heidelberger (New York), **Opener.**

An analysis of some of the more recent work on antigenic structure and the influence of this structure on the nature and combining properties of the corresponding antibodies was presented.

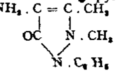
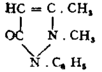
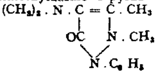
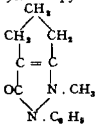
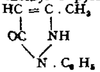
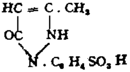
The present status of the specific polysaccharides was discussed and mention was made of the probable importance of the recurrence of specific groupings, or multivalence, both in antigens and antibodies.

Fully reported in Science, *1936*, **34,** *498*

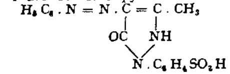
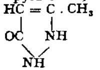
Dr. E. Berger (Basel) : *Beziehungen zwischen immunochemischen und andern Reaktionen.*

In der Einleitung wird darauf hingewiesen, dass das Bestehen gewisser Gemeinsamkeiten serologischer Reaktionen mit denen anderer, insbesondere mit denen der Fermente, schon seit langem angenommen wurde. Aber erst durch die Arbeiten Landsteiners u. Mitarbeiter über die chemospezifischen Antigene war die Möglichkeit eröffnet, die Analogien zu präzisieren.

Zunächst wird ein Paradigma gebracht, welches zeigen soll, dass bestimmte Aenderungen im Molekül, wie die Einführung der Acetylgruppe, die Spezifität sowohl künstlicher wie natürlicher Antigene beeinflusst. Landsteiner hatte schon in seinen früheren Arbeiten über Complexantigene festgestellt, dass gerade die Acetylierung die Spezifität bestimmter Anilinderivate verändern kann. Dieser Befund wurde durch die Untersuchungen von Enders und Chao-Jen-Wu sowie vor allem von Avery, Goebel und Babers über die Kohlenhydrate der Pneumokokken und über künstliche Glykoside vertieft, in welchem Zusammenhang vom Vortragenden die Studien von Freudenberg und Eichel über den Einfluss der Acetylgruppe auf die Specifität der Polysaccharide der Blutgruppensubstanz gestreift werden. Es wird dann gezeigt, dass auch auf einem ganz anderen Gebiete, nämlich bei den Glykosiden verschiedener Digitalisarten, die Unterscheidung der Digitalis-Glykoside durch die CH_3CO-Gruppe bedingt ist.—Eigene in Gemeinschaft mit Erlenmeyer an *Pyrazolonderivaten* ausgeführte Untersuchungen zeigten eine Uebereinstimmung zwischen pharmakologischer und serologischer Spezifität (Tabelle).

Verbindung	Reaktion mit Antiserum gegen Amido antipyrin	Antipyretisches Verhalten
Amido antipyrin = 1- Phenyl- 2, 3- dimethyl- 4- amino- 5- pyrazolon 	+	+
Antipyrin = 1- Phenyl- 2, 3- dimethyl- 5 pyrazolon 	+	+
Pyramidon = 1 Phenyl- 2, 3- dimethyl- 4 dimethylamino- 5 pyrazolon 	+	+
Trimethylene-*cyclo*-antipyrin 	+	+
1- Phenyl- 3- methyl- 5- pyrazolon 	—	—
1- Phenyl- p- Sulfosäure 3- methyl- 5- pyrazolon 	—	—

(Continued on next page

Verbindung	Reaktion mit Antiserum gegen Amido antipyrin	Antipyretisches Verhalten
1- Phenyl- p- Sulfosäure- 3- methyl- 4- azo- benzol- 5- pyrazolon 	—	nicht bekannt
3- Methyl- 5- pyrazolon 	—	,, ,,

Verf. geht dann auf die Bedeutung der "Feldwirkung" für die übereinstimmende serologische und physiologische Wirkung verschiedener Verbindungen ein, wobei als Beispiele Derivate von Thiophen und Benzol genannt werden, die sich isomorph im Kristallgitter vertreten, die im immunologischen Experiment in Uebereinstimmung mit Landsteiner und v.d. Scheer nicht zu differenzieren waren und die sich auch im pharmakologischen Versuch ähnlich verhielten.

Im Anschluss daran wird die Frage aufgeworfen, inwieweit man auf Grund der Formel der vorliegenden Verbindungen eine Parallelität der Wirkung bei verschiedenartigen Reaktionen erwarten kann. Ausgehend von den Versuchen über die Bedeutung der Feldwirkung kommt der Vortragende unter Berücksichtigung einer Arbeit von Grimm, Gunther und Tittus über die isomorphe Vertretbarkeit nichtpolar gebundener Atome und Atomgruppen zu dem Ergebnis, dass auch mit Hilfe moderner Vorstellungen über Atome und Molekül keineswegs bindende Schlüsse aus dem Formelbild gezogen, sondern nur Anhaltspunkte gewonnen werden können.

Schliesslich wird ein in methodologischer Beziehung erwähnenswerter Beitrag vorgelegt, der sich auf die Erforschung der Natur der Antikörper bezieht. Dabei werden Versuche angeführt über die mögliche Reinigung der Antikörpern durch spezifische Adsorption von Antisera an wasserunlösliche Haptene.

Dr. W. T. J. Morgan (Elstree, England) : *Is there Evidence for the Existence in a Single Bacterial Polysaccharide of Separate Antigenic Determinants of Diverse Specificity ?*

An examination by Meyer and Morgan of the pure specific polysaccharide of *B. dysenteriæ* (Shiga) has shown that it functions in two ways : (1) in combining with the homologous agglutinating and precipitating immune-substance, and (2) in neutralizing specifically the " Shiga " heterophile antibody. Since these specific combinations are independent reactions it appears that the single polysaccharide complex contains within its molecule two reactive and immunologically distinct structures, each of which is able to engender and react with its corresponding specific immune-substance Rabbits immunized with an artificially prepared " Shiga " polysaccharide-protein antigen, have been found to produce specific " Shiga " agglutinins and heterophile antibody.

These observations, when considered together with those made by Landsteiner and by Burnet indicate that a single bacterial polysaccharide is capable of functioning as an antigenic determinant of diverse specificity and it is suggested that, in an immune serum, the specificity of which is largely determined by a polysaccharide hapten, it is possible but not essential, that as many different kinds of antibody exist as there are individual antigenic structures in the polysaccharide molecule. Whether all of the antibody molecules that are engendered possess specific receptors for all of the antigenic structures in the polysaccharide must depend on the relative influence of the individual chemical structures concerned on antibody production.

Evidence has already been obtained which shows that certain chemical groups, owing to their special physical and chemical properties, play a dominant part in antibody formation and that they engender immune globulin molecules with which they combine specifically. It is believed that such active antigenic determinants will induce the formation of their corresponding immune receptors on *all* of the antibody molecules formed, whereas the less active antigenic structures that co-exist in the same polysaccharide molecule will cause their own receptors to appear less readily.

If the antigenic structures of a polysaccharide molecule are designated A, B, and C, and are arranged in order of their capacity to engender specific receptors on the immune globulin molecules, A being considered the dominant antigenic determinant, then the structures A, B, and C, will give rise to three main types of antibody molecules which will possess receptors corresponding to the following antigenic determinants,

a, ab, and abc.

Early during immunization with the antigen, antibody molecules will contain receptors for the most active antigenic structures only, whereas more prolonged immunization will elicit antibody molecules which possess receptors for most or all of the antigenic structures

of the polysaccharide. The less active antigenic structures may also occur in certain other immunologically active polysaccharides where they will give rise to cross-reactions. It is considered improbable that immune globulin molecules possessing a single kind of receptor corresponding with a minor antigenic structure, such as b or c, will be formed.

Some recent observations of Landsteiner suggest that a single determinant group may, owing to the influence of other antigenic structures in the same molecule, give rise to antibodies which differ somewhat in specificity and in combining affinity. Such antibody molecules will be able to react with similar but not identical antigenic structures in heterologous polysaccharides and will give rise to the phenomenon of overlapping specificity.

The theory that a pure bacterial polysaccharide, by virtue of the existence of a number of immunologically active structures in its molecule, is capable of fulfilling the function of more than one hapten, offers an alternative explanation to the one generally accepted, of the antigenic structure of those bacteria, for example, Flexner's dysentery bacillus and certain members of the Salmonella group of organisms, which are known to induce the formation of more than one antibody in the serum of animals immunized with them, and which have usually been considered to possess separate intracellular antigens corresponding to the antibodies formed.

Dr. W. F. Goebel (New York): *The Synthesis and Chemo-immunological Properties of Uronic Acid Antigens.*

Artificial carbohydrate-protein antigens, prepared by combining the diazonium derivatives of the p-aminobenzyl glycosides of glucose and glucuronic acid with foreign proteins, give rise in rabbits to antibodies which are immunologically distinct and specific. The uronic acid constituents of the type-specific polysaccharides of encapsulated micro-organisms are believed to have an important function in determining specificity. In support of this view it has been found that the glucuronic acid-protein antigen reacts in dilutions of 1 part in 1 million with antipneumococcus sera Types II, III and VIII, whereas the corresponding glucose antigen is serologically inactive. The precipitation of the artificial antigen in antipneumococcus sera may be attributed to the interaction of the glncuronic acid radical of the azoprotein with antibodies elicited by the bacterial polysaccharides containing similar uronic acid groupings. In this respect the artificial glucuronic acid-protein antigen bears a striking immunological similarity to certain of the encapsulating pneumococcus polysaccharides. Antisera thus far produced by immunization with the glucuronic acid-protein antigen have failed to afford protection against experimental pneumococcus infection. The reason for this may lie in the fact that the glucuronic acid radical of the artificial antigen does not approximate closely enough in chemical constitution to the uronic acid nucleus of the

specific bacterial polysaccharides, the aldobionic acids. For the preparation of artifical aldobionic acid-protein antigens it is necessary to obtain these acids either by isolation from natural sources, or by chemical synthesis. Two aldobionic acids, gentiobiuronic acid (glucose-6β-glucuronide) and galactose-6-β-glucuronide, identical with the naturally occurring aldobionic acid derived from the immunologically active gum acacia, have been prepared synthetically in our laboratory. Aldobionic acid antigens, in which the uronic acid nucleus approximates more closely to that of the bacterial polysaccharides, are now being prepared by synthesizing the appropriate aminobenzyl glycosides of these disaccharides.

Prof. J. R. Marrack (London) : *Cross Reactions between the Antiserum to Type II Pneumococcus and Various Gums.*

The antiserum to an antigen A may react with another antigen B, giving precipitation or agglutination ; in many cases all the antibody to A cannot be removed by the heterologous antigen B.

This may be accounted for by two hypotheses :

(I) Antigen A contains two or more determinant groups (*a*), (*b*), (*c*)— — —, and antigen B contains determinant groups (*a*), (*p*), (*q*)— — —. Antigen B only removes the antibodies to group (*a*).

(II) Antigen A contains a determinant group (a_1) and antigen B a determinant group (a_2) similar to but not identical with (a_1). The antibody molecules to group (a_1) differ in ability to combine or precipitate with (a_1). Only those more suited to (a_2) will be removed by antigen B. There is considerable evidence that antibodies to the same determinant group present in a given serum are not uniform.

Horse antisera to Type II pneumococcus form precipitates with gum acacia, gum ghatti, plum gum, cherry gum, mesquite gum, apricot gum.

(1) These reactions show the zone phenomena.

(2) If a serum is precipitated with gum acacia (or cherry gum) and the supernatant precipitated with Type II polysaccharide, the total antibody nitrogen precipitated by these two steps is equal to the total amount precipitable by Type II polysaccharide alone.

(3) The amounts of antibody nitrogen precipitable by the various gums differ.

(4) The salts of glucuronic acid and of glucuronides inhibit precipitate formation by the antiserum and the heterologous gums. These salts also delay and reduce the precipitate formation by antisera and Type II polysaccharide. These reactions are not delayed by salts of trimethyl glucuronic acid, galacturonic or mannuronic acids ; nor by glucose or galactose.

(5) A compound formed by coupling diazotised amino-euxanthic acid (amino-euxanthone glucuronide) with horse-serum proteins also forms a precipitate with Type II anti-seru (not with Type I

antiserum). This precipitation is also inhibited specifically by salts of glucuronic acid and glucuronides.

(6) If Type II serum is precipitated by this compound or by cherry gum, the reaction of the antibody, left in the supernatant, with Type II polysaccharide is still delayed and reduced by salts of glucuronic acid.

(7) Other evidence indicates that Type II serum does not contain antibodies to two different antigens, one of which antibodies is precipitated by cherry gum.

(8) All these gums contain uronic acids ; gum acacia—glucuronic acid, cherry gum—glucuronic acid ; mesquite gum—methoxy-glucuronic acid ; gum ghatti—mannuronic acid ; apricot gum—an acid like glucuronic acid, not galacturonic.

The data fit hypothesis (II) ; and suggest that the determinant group of Type II polysaccharide is glucuronic acid, or related to glucuronic acid.

Prof. F. Haurowitz (Prague): *Immunisierung mit Antigenen verschiedenen Arsengehaltes.*

Pferde-Serumglobulin wurde mit steigenden Mengen diazotierter Arsanilsäure (nach Landsteiner) gekuppelt. Die gebildeten Arsanil-azo-globuline enthielten 0·1 – 6·5 prozent As, das sind 1 bis 54 As-haltige determinante Gruppen pro Molekül (Molekulargewicht-100,000). Mit diesen Antigenen wurden Kaninchen immunisiert und die Immunsera kreuzweise ausgewertet. *Ergebnisse*: (1) Bei Immunisierung mit Antigenen kleinen und mittleren As-Gehaltes entstehen Präzipitine gegen natives Pferdeglobulin und Präzipitine gegen Arsanil-azo-eiweiss ; sie lassen sich voneinander trennen ; Präzipitine, welche sowohl Pferdeglobulin als auch Arsanil-azo-eiweiss präzipitieren, liessen sich nicht nachweisen. (2) Immunisierung mit Antigenen hohen As-Gehaltes (>2 prozent As) liefert nur schwach präzipitierende Sera : die As-reichen Antigene werden im Organismus nur schwach gespeichert, wahrscheinlich wegen ihres stark sauren Charakters. (3) Anti-arsanil-präzipitin präzipitiert nur Arsanil-azo-globuline mit > 5-10 determinanten Gruppen pro Molekül ; Arsanil-azo-globuline mit < 5-10 determinanten Gruppen werden durch das Präzipitin gegen Pferdeglobulin präzipitiert. (4) Arsanil-azo-globuline mit hohem As-Gehalt binden bei der Präzipitation mehr Antikörper als Arsanil-azo-globuline mit geringem As-Gehalt.

Die Versuche sprechen dafür, dass Antigen und Antikörper im Präzipitat ein alternierendes Aggregat bilden—wie Marrack dies angenommen hat ; jedes Antigen-Molekül haftet an mehreren Antikörper-Molekülen und jedes Antikörper-Molekül an mehreren Antigen-Molekülen, ähnlich den Ionen im Gitter eines Salzkristalls. Die Bildung eines solchen Aggregates ist nur möglich, wenn jedes Antigen-Molekül mehrere determinante Gruppen besitzt und jedes Antikörper-Molekül mehrere bindende Gruppen.

Dr. A. Wormall (Leeds) : *The Immunological Specificity of Chemically-altered Proteins.*

Phenyl *iso*cyanate and benzylcarbonyl chloride react with proteins, under mild conditions, to produce protein derivatives with a new immunological specificity. The action of these reagents on the protein appears to be confined almost entirely to a reaction with the free amino-groups (i.e., mainly the ε-amino-groups of lysine), and the new specificity is due to these phenylcarbamidz or benzylcarbonate groupings introduced. (Investigations with S. J. Hopkins and W .E Gaunt.)

Mustard gas, and the corresponding sulphone, also react with proteins to produce derivatives with a characteristic specificity, possibly by reaction with the free amino-groups of the protein. (Investigations with I. Berenblum.) The significance of these and related observations on the specificity of protein antigens was discussed.

Dr. L. D. Felton and **Mr. B. Prescott** (Baltimore, U.S.A.) : *Studies on the Chemistry of the Essential Immunizing Antigen of Type I Pneumococcus.*

A sample of Type I pneumococcus polysaccharide was prepared by the calcium phosphate method and purified by precipitation of the inert material with 2 normal $NaNO_3$ in the presence of 0·1 normal HCl, precipitating the soluble fraction with alcohol, and finally washing and drying the polysaccharide (Fraction A). In concentration of 1 mg. per c.c. in 0·1 normal NaOH, a sample was heated at 100° C. for 15 minutes, and then precipitated with alcohol and ether without neutralization (Fraction B). A fraction of this material was then treated with 25 per cent. NH_4OH for 24 hours at 4° C., and thrown down with alcohol and ether (Fraction C). The three samples were then tested chemically and immunologically as follows : acetyl was 7·3 per cent. in A, and down to 0·8 per cent. in B and C ; *alkoxyl 2·9 per cent. in A and negative* in B and C ; glucose number after hydrolysis was 15·6 per cent. in A and negative in B and C ; nitrogen content in all samples, even the one treated with ammonia, was the same, 4·4 per cent. The precipitin titre of the original A was 1:640,000, of the alkali treated B 1:80,000 and of the ammonia treated alkali fraction C up again to 1:2,500,000. Active immunity was respectively, A—1:25,000,000, B—1:1,000,000 and C—1:100,000,000. Optical rotation dropped from $[a]$ D = +150 in the original A to negative in the NaOH treated, and returned to $[a]$ D = + 170 in the ammonia alkali treated sample C. In addition, it was found that replacing the hydrogen of the hydroxyl groups, although markedly altering the precipitating power, did not destroy the immunizing activity. Our results indicate definitely that neither the acetyl nor the aldehyde group, nor protein is essential in producing active immunity in white mice. Our evidence that protein is not an intrinsic element is fourfold :

(1) negative biuret, (2) negative sulphur, (3) the general knowledge that hot alkali destroyed antigenicity, and (4) the reversibility of sample B, which is non-antigenic, into sample C, which is very highly antigenic, by means of ammonia. So far as analyzed, the immunizing activity of this antigen is associated with a polyhexuronic acid, and consqeuently is due, not to any particular side chain, but to a basic molecular structure.

Dr. I. Forest Huddleson and **Dr. R. Pennell** (East Lansing, U.S.A.): *The Chemical Constitution and Biological Activity of the Toxic Fraction of Brucella Cells.*

Material prepared from tryptic digest of Brucella cells; digest treated with 3 per cent. trichloroacetic acid for three hours, centrifuged, supernatant dialysed and dialysed fluid concentrated at 4° C. in vacuo to small volume. Purified by repeated precipitation with 10 volumes of alcohol in presence of acetic acid. Final product forms heavily opalescent solutions in distilled water.

Chemical Properties.—Fractions from *Br. abortus*, *Br. melitensis* and *Br. suis* are grossly similar. Suspensions in water slightly acidic; forms opalescent solutions in water made feebly alkaline; soluble in trichloroacetic acid; insoluble in alcohol slightly acidified with acetic acid. Gives weakly positive reaction with Millon's, biuret, Molisch and Bial's reagents. Nitrogen, 6 to 8 per cent. Yield 4 to 12 per cent. reducing sugars on hydrolysis (calculated as glucose). Not digested by enzymes. Phosphorus and sulphur absent. Acetone extraction yields an oil (? a diketone) ether and chloroform extraction yields a fat and an alcohol. Properties of residue, after fat extraction, substantially unchanged. Acid hydrolysis of fat-extracted residue gave a precipitate, negative to quantitative tests, and a supernatant fluid giving a strong Molisch reaction; from the hydrolysis product an acid (nitrogen-free) a crystalline substance containing all the nitrogen, glucose and arabinose were isolated. The purified toxic substance is not protein; the evidence suggests it is a complex polypeptide. The fractions from *Br. abortus* and *Br. melitensis* are not composed of all the same constituents.

Biological Properties.—Intraperitoneal injections of 20 mgm. doses in guinea-pigs cause fall in temperature, increased respiration, dullness and coma, death in 6 to 18 hours. Post-mortem findings: hæmorrhages in stomach mucosa, caecum, intestines, peritoneum, reproductive organs and glands: marked fall in leucocyte count involving chiefly granulocytes.

1-1,000 dilution of the fraction injected by various routes stimulate production of agglutinins, precipitins and immune opsonins. 1-200,000 dilutions of antigen precipitate immune sera.

1-2,000 dilutions elicit a local allergic reaction in Brucella sensitized human beings which is not produced in normal persons.

The efficacy of the fraction in active immunization is now being investigated.

Prof. M. Heidelberger, Dr. A. O. Menzel (New York) and **Dr. H. W. Scherp** (Philadelphia) discussed Bacterial Proteins.

Mr. P. Bruce White (London) : *Q-Proteins of Bacteria.*

From various Gram-negative bacteria (*Salmonella*, dysentery, coliform, *B. proteus*, vibrios) a peculiar protein fraction (Q-fraction) is readily extracted with a mixture of absolute alcohol (95 parts) and N/1 HCl solution (5 parts). The proteins are precipitated from the alcoholic solution on neutralization. The Q-substances are active antigens, stimulating the formation of agglutinins and precipitins which are widely group specific. Such antigens have not yet been isolated from Gram-positive bacteria, from fungi, or from plant and animal tissues.

Prof. E. K. Rideal and **Mr. J. H. Schulman** (Cambridge) : *Interactions in Monolayers.*

By measuring phase boundary potentials and surface pressures in monolayers on water it has been found that complexes formed by molecular association can be identified. This association is of two well-defined types, consisting of polar/dipole interaction and hydrophobic adhesion between the non-polar portion. The complexes are characterized by their strong stability when in monolayers, and to the apparent specificity to slight alterations in the polar or non-polar portion of one of the two interacting molecules.

If under the film of one component of associating molecules, the other component be injected, penetration of the film-forming substance takes place. This results in a large rise in surface pressure and the surface potential rises or falls to that of an equimolecular mixture. All those substances which penetrate cholesterol and disperse protein films are hæmolytic agents, and all those which are only adsorbed by protein films agglutinate or sensitize red cells. Association between cholesterol and lipins with proteins can be measured by these methods. The association is found to be sensitive to environment such as pH around neutrality.

Efforts are being made to utilize these methods to the study of the antibody/antigen reaction, by spreading in a suitable form the antibody and injecting the antigen or hapten into the underlying solution.

Dr. W. F. Goebel (New York) : *Studies on the Chemistry of the Essential Immunizing Antigen of Type I Pneumococcus.*

In contrast to the observations of Dr. Felton and Mr. Prescott, we found some years ago that the specific polysaccharide of Type I

pneumococcus induces type specific active immunity in mice only when the labile acetyl groups of the carbohydrate remain intact. On treating the acetyl polysaccharide with dilute alkali, the acetyl groups are entirely removed, and the resulting de-acetylated carbohydrate was found to be quite incapable of stimulating the production of type specific anti-pneumococcus immunity in mice. In view of these experiments one cannot deduce, therefore, as has Dr. Felton, that the acetyl groups of the specific polysaccharide of type I pneumococcus are unessential in rendering the carbohydrate antigenic in mice.

Dr. E. Berger (Basel).

(*a*) Zu den Ausführungen von Herrn Dr. Heidelberger möchte ich mir die Anfrage erlauben, inwieweit sich die immunisierenden Qualitäten der aus den Pneumokokken isolierten nicht desacetylierten Polysaccharide von den desacetylierten Produkten unterscheiden, wie sie früher erhalten wurden. Ich kann mir auch auf Grund der neueren Arbeiten von Avery, Goebel u. Francis kein klares Bild machen, ob ein eindeutiger Unterschied in Bezug auf das aktive Immunisierungsvermögen zwischen den beiden Produkten vorhanden ist. Soviel ich weiss, ist nur bei weissen Mäusen und beim Menschen eine aktive Immunisierung mit der Erzeugung schützender Antikörper mit den Acetyl-Polysacchariden geglückt. Ich wäre Herrn Dr. Heidelberger dankbar, wenn er mir sagen könnte, ob und inwieweit man in Parallelversuchen das Immunisierungsvermögen der beiden Polysaccharide verglichen hat.

(*b*) Auf die Frage von Herrn Dr. Heidelberger, ob die in unsern Untersuchungen als inaktiv gefundenen Pyrazolonderivate überhaupt keine Reaktionsfähigkeit mit Immunsera besitzen, möchte ich erwidern, dass wir die betr. Verbindungen nur mit den Antisera gegen 4-Amido-antipyrin ausgewertet haben, wobei die erwähnten Befunde erhalten wurden. Es ist aber nach allen bisherigen Erfahrungen mit chemospezifischen Antigenen nicht einzusehen, warum die Areaktivität der betr. Pyrazolonderivate eine generelle oder unspezifische sein sollte. Im übrigen darf ich auf die genauere Mitteilung im Archiv f. exp. Pathologie u. Pharmakologie 177,116.1934 verweisen.

Prof. M. Macheboeuf and **Mme. J. Blass** (Paris) : *Extraction de protéides solubles dans l'acétone à partir des vibrions cholériques.*

L'observation de Mr. P. Bruce White est très intéressante, elle peut être confirmée par le fait suivant que nous avons pu constater. Si l'on traite des vibrions cholériques par de l'acétone, à froid, on extrait naturellement des lipides, mais on extrait en outre de grandes quantités de protéides solubles dans l'acétone. Ces protéides sont solubles dans l'eau et insolubles dans l'éther ; ils représentent environ 10 pour 100 du poids total des vibrions cholériques secs.

De tels protéides solubles dans les solvants organiques doivent être éliminés avec soin lorsque l'on veut conclure à l'activité biologique des fractions lipidiques extraites des bactéries et il faut remercier Mr. Bruce White d'attirer aujourd'hui l'attention sur eux. Notons que l'acétone doit moins dénaturer ces protéides que l'alcool acide utilisé par Mr. Bruce White.

Prof. M. Heidelberger (New York), expressed himself as greatly encouraged in that several of the workers who had reported, in approaching similar problems often from opposing points of view, appeared to be converging toward a common understanding and interpretation of fundamentals. The use of absolute, quantitative methods to replace the older relative and qualitative ones was also increasing. He sounded a note of caution in drawing conclusions from cross-reactions, which often showed far-reaching quantitative and qualitative differences from the homologous reactions, and which, according to Landsteiner's most recent findings, could occur between antigens with unrelated haptens.

JOINT DISCUSSION *with* Section 1 *on* ANAEROBIC BACTERIAL METABOLISM

Chairman : Prof. A. J. Kluyver (Delft).

Dr. P. Fildes (London), **Opener** : *A Survey of the Anaerobic Field.*

Anaerobic growth is represented as a type of metabolism in which the H-acceptor in the energy-producing reaction is some substance other than oxygen. The oxidizable substance is oxidized by withdrawal of hydrogen to the acceptor which is reduced. Fumarate is an example of an H-acceptor for some facultative anaerobes (Quastel) and glycine or proline for *Cl. sporogenes* (Strickland).

The reaction between acceptors and donators probably takes place through a link (bio-indicator) which is contained in the cell and is reduced by the donator and reoxidized by the acceptor. The Eh of the link must lie between those of the oxidizing and reducing systems. Thus, a bacterium cannot obtain energy for anaerobic growth unless the Eh at its enzyme surfaces is appropriate to the link and systems involved. The Eh of the environment has no necessary bearing on the question. The cell has a *capacity* to maintain its Eh at a lower level than that of the environment. The environment can only stop anaerobic growth when its oxidizing capacity, exerted through diffusible substances, is able to overcome the reducing capacity of the cell and so interfere with the Eh of the cell which is appropriate to its energy-producing reactions.

The stimulation of anaerobic growth obtained by the use of sulphur-containing reducing substances, added with the idea of

reducing the medium may in some cases be due to this reduction, but also there is a specific SS—SH effect. These compounds are not only necessary for the growth of obligate anaerobes but also for the aerobic or anaerobic growth of *Staphylococcus aureus*. They have no special relation to anaerobiosis. A low Eh in the cell and the presence of reduced S compounds are characteristics of cells growing either anaerobically or aerobically.

Prof. H. Braun (Istanbul): *Ueber den anaeroben Verwendungsstoffwechsel der darmpathogenen Bakterien.*

Bei der fakultativen Anaerobiose sind folgende Tatsachen zu berücksichtigen:

1. Die Bakterien besitzen ein *individuell* wechselndes Fermentbildungsvermögen. Die Untersch ede in der Fähigkeit, Fermente zu bilden, können endogen oder exogen bedingt sein.

So finden sich z.B. unter den Coli-Bacillen Stämme, die mit NH_4Cl bei Traubenzuckeranwesenheit anaerob wachsen können, und andere, die unter diesen Bedingungen nicht gedeihen können. Ein Beispiel für die exogen bedingte Fermentbildung: Bei Fehlen von Schwermetallen in der Nährlösung ist die Bildung der Ameisensäuredehydrase der Flexner-Bacillen nicht möglich.

2. Für das anaerobe Wachstum ist die *Anpassung an die Substrate* von massgeblichem Einfluss.

Ein Beispiel: Flexner-Bacillen können, wenn sie aus einer aeroben Zucht im NH_4Cl-*Lactat*-Nährboden in einen Asparaginsäure + *Aepfelsäure* + Nitrat-Nährboden verimpft werden, in diesem nicht anaerob wachsen. Sie sind dazu aber fähig, wenn sie in diesem Nährboden von einer aeroben Kultur eines NH_4Cl + Aepfelsäure-Nährbodens eingeimpft werden.

3. Bei Abwesenheit von Sauerstoff ist der assimilatorische Stoffwechsel der Bakterien eingeengt: Keime, die unter aeroben Bedingungen inbezug auf Stickstoff- und Kohlenstoffquelle anspruchslos sind, werden bei Luftabschluss anspruchsvoll. Sie verlangen besonders gebaute Kohlenstoff- *oder* Stickstoffquellen, bzw. Kohlenstoff- *und* Stickstoffquellen.

Ein Beispiel: Paratyphus-B-Bacillen können sehr gut in einem NH_4Cl + Lactat-Nährboden aerob gedeihen, während sie zum anaeroben Wachstum Asparaginsäure als Stickstoffquelle benötigen. Ist Brenztraubensäure vorhanden, die eine bessere Kohlenstoffquelle ist als Traubenzucker, dann vermögen sie auch mit NH_4Cl unter Luftabschluss zu wachsen.

4. Die Wasserstoffionenkonzentration ist für das anaerobe Wachstum von Bedeutung. Alkalische Reaktion ist meist optimal.

5. Die Reduktionsintensität des Mediums ist für manche fakultativen Anaerobier, wie Streptokokken und Pneumokokken, von Bedeutung.

6. Schwermetalle müssen in geringer Menge für die Bildung mancher zum anaeroben Wachstum wichtiger Fermente, z.B. der

Dehydrasen, anwesend sein. Besonders geeignet für die Bildung der Dehydrasen ist die "tryptic broth" von *S. W. Cole* und *H. Onslow* oder die übliche Fleischwasserpeptonbouillon mit Zusatz von Valin, Leuzin, Lysin, Zystein, Tyrosin, Hystidin und Prolin.

7. Die Konsistenz des Mediums ist vielleicht für den anaeroben Stoffwechsel von Einfluss. Flüssige Medien scheinen den anaeroben Stoffwechsel zu fördern.

8. Kohlensäure kann das aerobe und anaerobe Wachstum begünstigen. (*Walker, Gladstone, Fildes* und *Richardson*).

9. Zur Vermehrung mancher fakultativen Anaerobier, z.B. Staphylokokken, aber auch wahrscheinlich anderer Keimarten, sind akzessorische Wuchsstoffe erforderlich. (*Hughes, Hirsch und Muller, Fildes* und *Knight*).

***Prof. W. Frei** (Zürich) : *Die Sauerstoffaufnahme von ruhenden aeroben und anaeroben Bakterien.*

Eine alte Erfahrung besagt, dass die anaeroben Bakterien durch Sauerstoff derart geschädigt werden, dass sie wenigstens bei der Sauerstofftension der Atmosphäre (ca 160 mm. Hg.) nicht zu wachsen vermögen. Andererseits wird berichtet, dass das Sauerstoffoptimum für die obligaten Anaerobier nicht Null, sondern 3-15 mm. Hg. beträgt.

Wir untersuchten im Warburgapparat die Sauerstoffaufnahme durch gewaschene, streng anaerobe und vergleichsweise auch durch obligat aerobe und fakultativ anaerobe Bakterien.

Nach allerlei Vorversuchen, welche den Einfluss des Alters der Kultur, des Waschens, des Schüttelns u.s.f. zum Ziele hatten, wurden Versuchsreihen mit obligaten Aerobiern und fakultativen und obligaten Anaerobiern durchgeführt.

Ein besonderer Unterschied in der Sauerstoffaufnahme von obligaten Aerobiern und von Halbanaerobiern besteht nicht. Hingegen ist bemerkenswert, dass einige Arten der sogenannten Anaerobier nämlich Putrificus, Amylobacter, Welch-Fraenkel, Bazillus des malignem Oedems und auch noch der Rauschbrandbazillus in ruhendem Zustande Sauerstoff aufnehmen. Der Grössenordnung nach liegen die von Putrificus und Amylobacter aufgenommenen O_2-Mengen unter denjenigen der obligaten Aerobier und auch der meisten Halbanaerobier. Unter diesen letzteren besitzt aber der Mastitisstreptococcus (wahrscheinlich auch der Rotlaufbazillus) ein sehr geringes Sauerstoffaufnahmevermögen, welches nicht über dasjenige des anaeroben Putrificus hinausgeht. Eine Sauerstoffaufnahme konnte beim Novy-, Tetanus- und Botulinusbazillus nicht nachgewiesen werden.

Nach unseren früheren Untersuchungen fehlen die Oxydationssubstanzen Cytochrom und Oxydase den Milchsäurestreptococcen, dem *Strept. equi* und einem haemolytischen Streptococcus. Diesem letzteren fehlt auch die Katalase während der Peroxydasegehalt

wechselnd ist. Der *Strept. mastitidis* besitzt von allen Oxydationsenzymen kein einziges. Beim Bazillus des Schweineerysipels fanden wir nie Cytochrom, bei einigen Kulturen Oxydase und Peroxydase, bei andern nicht. Regelmässig war nur das Vorkommen von Katalase.

Das Fehlen von scharfen Grenzen zwischen der Gruppe der sogenannten strengen Aerobier einerseits und den Halbanaeroben und strengen Anaeroben andererseits wird durch unsere Sauerstoffaufnahmeversuche neuerdings offenkundig.

Durch unsere Experimente wird dargetan, dass das Fehlen jeglicher Oxydationsfermente nicht gleichbedeutend ist mit dem Unvermögen der Sauerstoffaufnahme.

Dr. G. M. Richardson (London): *The Anaerobic Growth of* Staphylococcus aureus.

The hydrogen donators and acceptors which can be activated by *Staph. aureus* have been investigated by the Thunberg technique. The results have been correlated with the anaerobic growth requirements of this organism.

Of fifty compounds studied, six could be activated as acceptors (glyoxal, methylglyoxal, diacetyl, fumarate, pyruvate, and nitrate). Of these, only pyruvate could support anaerobic growth of *Staph. aureus* on a medium that was otherwise adequate. At least thirty compounds were found which could be activated as donators. Of these, only the sugars, sugar alcohols, and possibly amino-acids were of sufficient reducing intensity to donate to a weak acceptor such as pyruvate. In agreement with these findings, slow anaerobic growth with pyruvate could occur on amino-acids, but was greatly accelerated by addition of sugars or sugar alcohols (but of none other of the usual carbon substrates).

An accessory factor was found which was indispensable for anaerobic growth in presence of pyruvate, though unnecessary for aerobic growth. The possibility that failure of growth with acceptors other than pyruvate might be due to absence of their appropriate accessory factors was considered.

Dr. L. H. Stickland (Leeds): *The Amino-acid Metabolism of the Anaerobic Bacteria (Genus* Clostridium).

Anaerobes can grow on a medium containing practically nothing but amino-acids, and must therefore be able to obtain their energy from chemical reactions involving only amino-acids. The most likely type of reaction is one involving oxidation of one amino-acid molecule at the expense of the reduction of another.

Experimentally it was found that a number of amino-acids (especially alanine, valine and leucine) can, in the presence of a washed suspension of *Cl. sporogenes*, act as hydrogen donators to indicators, and others (proline, hydroxyproline and glycine) similarly

act as hydrogen acceptors. Also, even in the absence of indicators, washed suspensions of *Cl. sporogenes* are able to catalyse the linked reactions between hydrogen donators and hydrogen acceptors; for instance, if alanine and glycine are incubated together anaerobically with washed *Cl. sporogenes*, the alanine is oxidized and the glycine reduced.

Such reactions proceed at a rate of the same order as those of aerobic oxidations with other species of bacteria. The amount of energy liberated is unknown, but recent work of Fildes and Richardson increases the probability that these reactions supply all the energy required for the growth of *Cl. sporogenes*.

Proline and glycine in their reduction take up two atoms of hydrogen, giving respectively δ-amino-valeric acid and acetic acid and ammonia. Alanine on oxidation loses four atoms of hydrogen, giving acetic acid, carbon dioxide and ammonia.

Mr. D. D. Woods (Cambridge) : *The Amino-acid Metabolism of the Anaerobic Bacteria (Genus* Clostridium*).*

The work of Stickland on the coupled oxidation and reduction of pairs of amino-acids in the presence of suspensions of *Cl. sporogenes* has been extended. The amino-acid ornithine acts powerfully as hydrogen acceptor, oxidizing alanine, leucine, etc., in the presence of the organism. Ornithine alone is also partially broken down by active suspensions. On reduction in the presence of a hydrogen donator ornithine takes up two atoms of hydrogen, loses one molecule of ammonia and gives rise to δ-amino-valeric acid.

Cysteine is also attacked by suspensions of *Cl. sporogenes*, both ammonia and hydrogen sulphide being split off. Besides this action it is also activated as a hydrogen donator and reduces glycine or proline in the presence of the bacterium.

The amino-acid arginine also acts as H acceptor and brings about the oxidation of H donators. In addition it is deaminated to a considerable extent in the absence of any donator.

The relation between structure of the amino-acids and their power to act as donators or acceptors in the " Stickland " reaction was discussed.

***Dr. B. C. Guha** (Calcutta) and **Dr. N. Das** (Stockholm) : *The Respiration of* Staphylococcus albus *and* Staphylococcus aureus.

The presence of flavins in certain bacteria and the rôle of flavins in respiration, indicated by recent researches, led us to investigate the effect of lactoflavin on the respiration of *Staph. albus* and *aureus*, both aerobically and anaerobically cultivated. It was considered that the pigmented and non-pigmented varieties might show some significant difference in this respect. No stimulating effect of lactoflavin was, however, obtained in either case with alanine and glucose as substrates. It was, however, observed that in the

anaerobically cultivated organisms the proportion of cyanide-stable respiration to the total respiration was significantly greater than in the aerobically cultivated organisms. This proportion was also found to rise with prolongation of the period of cultivation. It seems, therefore, that in *Staph. albus* and *aureus* there is a certain cyanide-stable respiratory system which is not connected with the flavins.

Dr. A. R. Prévot (Paris).

Du point de vue de la systématique des bactéries, les communications de cette section attirent une question de grande importance. En dehors de la différence de métabolisme général concernant exclusivement le comportement des anaérobies vis à vis de l'oxygène, existe-t-il vraiment une démarcation nette entre le métabolisme général des anaérobies et celui des aérobies ? Si oui, on sera obligé de faire deux grands groupes de bactéries : d'un côté les aérobies, de l'autre les anaérobies. Si non, comme nous le pensons personnellement, il faut laisser les anaérobies répartis parmi les groupes naturels bactériens, ordres, familles, et genres, si bien qu'un vieux genre peut avoir à la fois des espèces aérobies et des espèces anaérobies.

Par conséquent dans la seconde alternative, il ne faut pas suivre la tendance actuelle, qui est de fonder des genres exclusivements aérobies ou exclusivement anaérobies. Cette tendance aurait, parmi d'autres inconvénients celui d'exclure de la systématique les bactéries primitivement anaérobies qui secondairement deviennent facultatives.

JOINT DISCUSSION *with* Section 1 *on* NUTRITIONAL FACTORS ASSOCIATED WITH THE GROWTH OF MICRO-ORGANISMS

Chairman : Dr. P. Fildes (London).

Dr. A. Lwoff (Paris), **Opener :** *Étude sur les fonctions perdues.*

Ce rapport a été limité à l'examen de quelques problèmes généraux posés par l'étude des facteurs de croissance qui sont définis ainsi : un facteur de croissance pour un organisme donné est une substance dont cet organisme est incapable d'effectuer la synthèse et en l'absence de laquelle toute multiplication de cet organisme est impossible.

Certaines bactéries sont capables de se multiplier dans un milieu synthétique, renfermant un sel d'ammonium comme unique aliment azoté. Ces bactéries réalisent donc la synthèse de tous les acides aminés constitutifs des proteides. D'autres ne se multiplient que si on leur fournit un ou plusieurs acides aminés. Ces acides aminés doivent être considérés comme des facteurs de croissance pour les organismes incapables d'en faire la synthèse.

Si l'on envisage la forme sous laquelle l'azote doit être fourni pour la synthèse des acides aminés, on constate que certains organismes peuvent utiliser NO_3 comme unique aliment azoté, tandis que d'autres ont besoin de NH_3. Il existe donc des organismes capables de réduire NO_3 en NH_3, et d'autres qui en sont incapables et pour lesquels NH_3 est un facteur de croissance.

Les organismes "euthiotrophes" sont capables d'utiliser les sulfates comme aliment sulfuré, c'est à dire de réduire SO_4 ; les organismes "parathiotrophes" en sont incapables et on besoin d'une source de soufre réduit—SS,—SH ou—CS (Volkonsky). De nombreux Phycomycètes sont incapables de réaliser la synthèse de la vitamine B_1 (Schopfer). Les organismes parahémotrophes (*Hæmophilus canis, H. influenzæ*) ne peuvent faire la synthèse de l'hématine—l'étude du flagelle trypanosomide de *Strigomonas fasciculata* montre que l'action de l'hématine est spécifique hématohémine, mésohémine, deutérohémine, phéophorbide-hémine, turacine, cytochrome C sont dépourvus d'action). L'action quantitative sur la multiplication est une des propriétés fondamentales des facteurs de croissance : au voisinage des doses limites le développement est proportionnel à la quantité de facteur de croissance présent dans le milieu. L'étude de la respiration montre que l'hématine est utilisée pour la synthèse de groupements prosthètiques des constituants respiratoires de Keilin-Warburg : cytochrome et cytochrome-oxydase. L'hématine n'agit donc pas comme catalyseur sur la multiplication, mais entre dans la constitution du système catalytique respiratoire. Le cytochrome C, dépourvu d'action, peut être utilisé par *S. fasciculata* et *Hæmophilus canis* en présence d'hématine. De nombreux exemples sont donnés du caractère fondamental des facteurs de croissance : les organismes qui n'en ont pas besoin pour se multiplier en réalisent la synthèse (facteur du bacille de Johne, de *Cl. sporogenes*, facteurs X et V). L'étude comparative de l'évolution morphologique des protozoaires et de leur pouvoir de synthèse a conduit à la conclusion qu'il existe une évolution physiologique caractérisée par des pertes successives de fonctions. Une évolution analogue existe aussi chez les bactéries (Knight)[1]. Conclusion : Le besoin d'un facteur de croissance correspond à la perte du pouvoir de synthètiser l'un des constituants fondamentaux de la matière vivante.

Prof. S. Orla-Jensen, N. C. Otte and A. Snog-Kjaer (Copenhagen) : *The Vitamin and Nitrogen Requirements of the Lactic Acid Bacteria.*

Lactic acid production by various bacteria is compared using normal milk and milk treated twice with activated charcoal and centrifuged for removal of charcoal.

[1] Bibliographie *in* Knight, B. C. J. G. Bacterial Nutrition. *Med. Res. Council*, 1936, *Spec. Rep. Ser.*, No. 210.

TABLE I.

Nutrient Medium	Species of bacteria		
	Thermo-bacterium lactis 10	*Strepto-bacterium casei* 11	*Strepto-coccus thermo-philus* 7
Carbon-treated milk—			
Without any addition ...	0·3	0·2	0·3
With lactoflavin	0·5	0·2	0·2
,, bios (eluate)	1·0	2·3	5·2
,, lactoflavin + bios ...	12·2	10·8	5·9
Normal milk	16·2	5·6	6·3

Elution of the charcoal showed that lactoflavin and a thermostable substance, probably bios, had been adsorbed.

The bacteria grew poorly on the carbon-treated milk and produced but little acid. The treated milk cannot be reactivated by addition of lactoflavin alone, but it is reactivated with Streptococci on addition of bios in the same amount as in cow's milk and with rod-shaped lactic acid bacteria by addition of lactoflavin + bios.

Streptococci also require lactoflavin but in the much smaller amounts of the eluate.

Propionic acid bacteria, many micrococci, the fluorescent bacteria and *Bact. prodigiosum* grow better and give more pigment with lactoflavin than without. On the other hand, the typical *Bact. prodigiosum* and *Bact. violaceum* give most pigment on the charcoal-treated milk. Here the pigments appear to play the same part as bios and when sufficient bios is present they are not produced. Coli and aerogenes bacteria thrive as well in charcoal-treated as in normal milk, in accordance with the fact that they are able to grow on synthetic media.

Peptone prepared from the usual acid caseinogen is a good, and from purified caseinogen a poor source of nitrogen for lactic acid bacteria, but the deficiency of the latter is remedied by addition of bios. Addition of lactoflavin is without effect. It appears, therefore, that caseinogen and Witte's peptone contain sufficient lactoflavin. That this lactoflavin is introduced during peptonization of the caseinogen along with the enzymes used is shown from the results in *Table 2*.

TABLE 2.

Nutrient Medium	Species of Bacteria	
	Thermo-bacterium lactis 10	*Strepto-bacterium casei* 11
Carbon-treated milk—		
Without any addition	0·1	0·7
With bios	0·8	2·7
,, ,, + lactoflavin 0·5 mg/l ...	11·5	11·7
,, ,, + impure pepsin 100 mg/l ...	11·0	11·7
,, ,, + pure ,, ,, ...	5·0	8·8
,, ,, + pancreatin ,, ...	9·9	12·2
,, ,, + impure papain ,, ...	8·6	10·4
,, ,, + pure ,, ,, ...	4·3	6·3
,, ,, + rennet solution ,, ...	12·8	13·7
Normal milk	14·4	5·4

All the proteolytic enzymes tested are rich in lactoflavin whether they are of plant or animal origin. The purer the enzyme preparation the less lactoflavin it contains. The crude enzyme preparations may contain 10,000 times the lactoflavin content of milk.

TABLE 3.

Nutrient Medium	Species of bacteria		
	Thermo-bacterium lactis 10	*Strepto-bacterium casei* 11	*Strepto-coccus cremoris* 18
Whey without any addition ...	0·2	0·2	0·9
Lactose solution + peptone Witte	2·1	0·6	2·6
,, ,, + caseinate ...	0·5	0·5	0·0
Whey + peptone Witte	9·4	9·6	5·6
,, + caseinate ...	9·5	10·4	5·4
Milk diluted to 0·2 per cent. N	14·4	8·1	4·5

Table 3 shows that protein-free whey is a very poor source of nitrogen although it is rich in activators. Colloidal caseinogen, dissolved in whey is just as good a source of nitrogen for lactic

acid bacteria as Witte's peptone, in spite of the fact that these bacteria do not excrete proteolytic enzymes. Dissolved in sugar solution (with nutrient salts) caseinogen and Witte's peptone have not the same effect as in whey because the sugar solution lacks the necessary activators.

Only the *Thermobacteria* require complete proteins or the entire complex of amino-acids therein contained. *Streptobacteria* develop with ammonium salts and a trace of cysteine and *Streptococci* with ammonium salts alone, if they have the necessary activators at their disposal. Rod-shaped lactic acid bacteria require, in addition to bios and lactoflavin, a third activator which is not adsorbed by charcoal but can be obtained by evaporation of whey along with the first fraction of lactose crystals.

The fact that among the lowest organisms some species require not less than three vitamins makes it likely that when a bacterium fails to grow on an artificial medium, the reason may be lack of some activator rather than of some nutrient.

Miss Relda Cailleau (Paris) : *Sterols considered as Growth Factors for the Flagellate* Trichomonas columbæ.

Trichomonas columbæ is normally grown in Tarozzi's medium (ordinary nutrient broth containing a fragment of liver). Experiments showed that the growth factors contained in the liver could be separated by acetone extraction.

The fraction found in the acetone extract could be replaced by cholesterol.

Various other sterols were studied in an attempt to correlate the activity of assimilation of the sterol with its molecular configuration.

The following results were observed :—

(1) Esterification of the cholesterol does not alter its activity.

(2) The double bond in position 5 : 6 is not essential and may be eliminated and replaced by a H in position 5.

(3) The alcohol function in position 3 may be replaced by a ketone.

(4) The shift of the double bond from position 5 : 6 in ring II to position 4 : 5 in ring I inactivates the sterol.

(5) Epimerization of the OH group in position 3 inactivates the sterol.

(6) Similar epimerization in position 4 or 5 of either the OH group present (as in trans-cis $\Delta 5:6$ cholestene 3 : 4 diol) or the H group (as in the coprosterols) inactivates the compounds. The *cis* position seems to be fatal to the activity of the sterol.

(7) Complete elimination of the lateral side chain inactivates the sterol.

(8) Modification of the side chain as seen in $\Delta 5:6$-3 oxycholenic acid is sufficient to inactivate the sterol.

(9) Slight changes in the structure of the side chain, as found in sitosterol, cinchol, and ergosterol do not affect the activity of the sterol.

This is thought to be the first example of an organism requiring cholesterol and being unable to synthesize it. It is believed that in many other cases where serum, egg tissue or other complex substance is necessary for the growth of an organism, the complex substance may, in part at least, be required because the cholesterol contained therein is one of the necessary growth factors for the organism.

Dr. B. C. J. G. Knight (London): *Bacterial Nutrition and the General Rôle of Certain Growth Factors.*

A comparison of the nutritional requirements of bacteria allows them to be arranged in a rough series showing increasing complexity of nutrient requirements. The simplest are the autotrophic bacteria and other soil bacteria (e.g., *Azotobacter*, *Ps. fluorescens* and *pyocyanea*). Then follow bacteria more intimately associated with animal life, whose nitrogen requirements tend to demand amino-acids rather than ammonia (e.g., Salmonella, cholera, dysentery and typhoid groups). Still more complex are the nutrients required by pathogenic bacteria whose relation to animals is approaching that of parasitism. With these, besides specific amino-acids and suitable carbon compounds to serve as available sources of nitrogen, carbon and energy, special substances are required as essential nutritional components. These may be referred to, for convenience, as bacterial growth factors, without prejudging their chemical nature or function, and using the term to embrace the more intimate nutrient essentials, which the organism cannot synthesize and yet requires for growth under the given conditions.

The above sequence of bacteria, going from those with great synthetic power and correspondingly simple nutrient needs to those whose nutrient requirements are complex, is suggested to be on the whole an evolutionary sequence showing increasing loss of synthetic power and consequent dependence on finding certain essential nutrients preformed in the environment[1]

It has been found that the "sporogenes" factor is required not only by *Cl. sporogenes*, but also by *Cl. botulinum* and by *Cl. welchii*.[2, 3] Similarly, the "staphylococcus" factor is important also for the growth of *B. anthracis*.[4] Both these factors are synthesized by many varied bacteria of the nutritionally simpler groups. The requirement of X and/or V factor by various species of the influenza group is related to their ability to synthesize these

[1] Knight, B.C.J.G.: "Bacterial Nutrition: Material for a comparative physiology of bacteria". Med. Resear. Coun. *Sp. Rep. Series* No. 210, 1936.
[2] *Brit. Jour. Exp. Path.*, 1933, 14, 112.
[3] *Ibid.*, 1935, 16, 309.
[4] *Ibid.*, 1935, 16, 315.

factors. The V factor is also synthesized by many other bacteria. It would be to suggest excessive purposiveness to suppose that all those bacteria which synthesize the various growth factors above mentioned did so only for the benefit of those bacteria which could not synthesize them, but required them; it seems more probable that those bacteria which synthesize these substances also use them in their metabolism.[6] These observations find a rational explanation in the hypothesis of an evolutionary development correlated with losses of synthetic power, which has played a rôle in the development of pathogenicity and parasitism. It may be found that those bacterial growth factors which have already been described as essential nutrient components for one or a few species actually play a general rôle in bacterial metabolism and are intiimately connected with metabolic reactions that are common to large groups of bacteria, which are differentiated among themselves, in that some species can synthesize these factors, while others have lost this power and therefore need to be given these substances.

Mr. J. R. P. O'Brien (Oxford): *Bios, the Yeast Growth Factor.*

Wildier defined bios as a chemical substance indispensable for the growth of yeast. Since 1901, investigation of the nutritive requirements of yeast has revealed that yeasts vary in their bios requirements. Some yeasts grow without bios whilst others thrive on one or more bios substances. In the bios group of substances may be placed the inositol and Bios II of Miller *et al.*, Pantothenic acid, and biotin of Kögl.

The growth of *Sacch. cerevisiæ* has been investigated using a medium containing adequate minerals (Fe, Cu, and Tl) and inositol, which may be regarded as a stimulant. A bios extract was prepared from cotton wool and yeast possessing an activity of $0 \cdot 5\ \gamma$ per c.c. medium. This extract has marked blue fluorescent properties which are not due to the presence of Kögl's biotin or thiochrome, and its bios activity is not destroyed by nitrous acid—a reaction differentiating it from pantothenic acid. Preliminary experiments indicate that bios may influence the budding rate and respiration of yeast.

Mr. C. G. Pope (Beckenham, England): *The Nitrogen Requirements of* Corynebacterium diphtheriæ *as affecting the Composition of Media for the Routine Production of Toxin.*

Certain observations which have been made on this question were discussed.

These dealt with :—

(1) The effect of dilution of tryptic broth by a " salt mixture solution " on growth and toxin production.

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[6] *Arch. Mikrobiol.*, 1935, 6, 139, 196, 209. *Z. Vitamin forsch.* 1935, 4, 67, 187.

(2) The separation of the medium into fractions, and the effect of varying the ratio of the fractions present in the medium on growth and toxin production.
(3) The effect of carbohydrates present in meat.
(4) The value of certain peptones for use in semi-synthetic media.

Prof. A. J. Kluyver (Delft).

One of the principal points which should be considered in the question of accessory growth factors seems to be to decide whether such factors should be considered to be of an absolute or of a conditional nature. It seems quite possible and even probable that some substance which is essential for the growth of a given micro-organism in a special medium may be quite superfluous if the same organism is inoculated into a medium of quite different composition. The deficiency observable in the first medium will be due to the circumstance that the organism is unable to synthesize some essential cell substance ; such a synthesis may well be possible when other substrates are offered to the organism. Especially it is likely that with one and the same species, the metabolic process also—fermentation or respiration—may well determine whether some substance is an indispensable growth factor or not.

Dr. A. Lwoff (Paris), queried Dr. Knight's suggestion that the complex nutritional requirements of parasitic bacteria might be due not only to loss of synthetic power, but also to the development of *new* growth requirements, needing substances that none of them normally synthesize, but which were perhaps characteristic products of the growth activities of other organisms.

Dr. B. C. J. G. Knight (London) replied that the host might possibly produce some characteristic *metabolic* product, which the parasitic organism used as nutrient. This product need not be synthesized by the host (e.g., an animal), or by the organism it used for nutrient (e.g., plants) although, of course, the compound from which the characteristic metabolic *product* came must have been. Thus, perhaps, a complex compound X used as nutrient by the animal might be broken down in the course of metabolism, giving a characteristic metabolic product Y derived from X. If other animals using X did not give Y, then the parasitic micro-organism which used Y would not be parasitic thereon.

Prof. W. H. Schopfer (Bern) : *Action du facteur du Staphylococcus sur Phycomyces.*

La vitamine B[1] ne peut remplacer le facteur du staphylococcus ; mais ce dernier peut avoir une faible activité B[1] sur *Phycomyces ;* il faut cependant tenir compte de l'intervention du facteur M que

nous avons découvert et qui est différent du bios et du facteur B[1]. Il est caracterisé par une très forte thermostabilité et est absorbé par le noir animal ; on ne peut donc dire avec certitude que le facteur du staphylocoque a une activité B[1]-spécifique sur *Phycomyces*. *Phycomyces* réagit spécifiquement lorsque la vitamine B[1] pure est présente seule, lorsqu'il s'agit d'un complexe de substances, il faut tenir compte de l'intervention possible du facteur M, qui semble très répandu.

DISCUSSION *on* METABOLIC PRODUCTS OF THE LOWER FUNGI

Chairman : Prof. H. Raistrick (London).

Dr. P. W. Clutterbuck (London), **Opener.**

During recent years, the study of the biochemistry of the lower fungi has shown that these organisms are capable of producing from simple carbohydrates a wide series of metabolism products. The following products were discussed in detail and specimens of most of them exhibited. They were conveniently grouped into the following classes, the organisms producing them being given in brackets in each case.

(*a*) *Metabolism products containing the same or smaller numbers of carbon atoms than the parent carbohydrate* :—

Oxalic acid (*A. niger*, etc.), ethyl alcohol (species of *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Fusarium*, etc.), acetaldehyde (species of *Mucor*, *Aspergillus*, *Penicillium*, etc.), ethyl acetate (*P. digitatum*), lactic acid (*Rhizopus* species) ; fumaric acid (*A. fumaricus*, *Rhizopus nigricans*, *P. griseo-fulvum*, etc.), malic acid (species of *Aspergillus* and *Clasterosporium*), succinic acid (*P. spiculisporum*, *A terreus*, species of *Mucor*, *Aspergillus*, *Clasterosporium*, etc.), γ-methyltetronic acid $C_5H_6O_3$ (*P. Charlesii*), itaconic acid $C_5H_6O_4$ (*Aspergillus itaconicus*), citric acid (*Citromyces* and many species of *Aspergillus* and *Penicillium*), *d*-gluconic acid (*P. purpurogenum*), *i*-erythritol (*P. brevi-compactum*), mannitol (white species of *Aspergillus*, *Byssochlamys fulva*, etc.), kojic acid, $C_6H_6O_4$, 5-hydroxy-2-hydroxy-methyl-γ-pyrone (*A. flavus*, *oryzæ*, *tamarii*, etc.).

(*b*) *Metabolism products containing a larger number of carbon atoms than the parent carbohydrate* :—

(1) *Benzenoid compounds* : Gentisic acid $C_7H_6O_4$, 2:5-dihydroxybenzoic acid (*P. griseo-fulvum*) ; 6-hydroxy-2-methylbenzoic acid $C_8H_8O_3$ (*P. griseo-fulvum*) ; mellein $C_{10}H_{10}O_3$ which gives 6-hydroxy-2-methylbenzoic acid on KOH fusion (*A. melleus*), a methoxy-dihydroxy-*p*-toluquinone, $C_8H_8O_5$ (*P. spinulosum*) ; puberulic acid, $C_8H_6O_6$, probably an aldehydotrihydroxybenzoic acid, and an acid $C_8H_4O_6$ (*P. aurantiovirens*, *P. puberulum*) ; mycophenolic acid $C_{17}H_{20}O_6$, 3:5-dihydroxy-2-carboxybenzylmethylketone $C_{10}H_{10}O_5$,

3:5-dihydroxy-2-carboxy-phenylacetylcarbinol $C_{10}H_{10}O_6$, hydrated 3:5-dihydroxy-2-carboxy-benzoylmethylketone $C_{10}H_{10}O_7$, 3:5-dihydroxyphthalic acid (*P. brevi-compactum*).

(2) *Heterocyclic compounds* : Carolic acid $C_9H_{10}O_4$, carolinic acid $C_9H_{10}O_6$, carlic acid $C_{10}H_{10}O_6$, carlosic acid $C_{10}H_{12}O_6$; these are butyrolactone, succinic and butyric acid derivatives of γ-methyltetronic and γ-tetronylacetic acids (*P. Charlesii*).

(3) *Crystalline pigments* : Citrinin $C_{13}H_{14}O_5$, a quinonoid acid (*P. citrinum*, etc.) ; citromycetin $C_{14}H_{10}O_7.2H_2O$, a dihydroxycarboxybenzpyrone (*Citromyces* species) ; Helminthosporin, $C_{15}H_{10}O_5$, 2-methyl-4:5:8-trihydroxyanthraquinone (*Helminthosporium gramineum*) ; catenarin, $C_{15}H_{10}O_6$, β-(hydroxymethyl)-1:5:8-trihydroxyanthraquinone (*H. catenarium*) ; cynodontin, $C_{15}H_{10}O_6$, 1:4:5:8-tetrahydroxy-2-methylanthraquinone (*H. cynodontis*) ; tritisporin, $C_{15}H_{10}O_7$, 1:3:5:8-tetrahydroxy-6-(or -7) hydroxymethylanthraquinone (*H. tritici-vulgaris*) ; auroglaucin $C_{19}H_{22}O_3$ and flavoglaucin $C_{19}H_{28}O_3$ (*A. glaucus* species) ; rubroglaucin $C_{16}H_{12}O_5$, a methoxyhydroxyanthraquinone (*A. glaucus* species) ; chrysogenin $C_{18}H_{22}O_6$, a hydroxyquinone (*P. chrysogenum*) ; ravenelin, $C_{14}H_{10}O_5$, a methyltrihydroxyxanthone (*H. Ravenelii*) ; fulvic acid, $C_{15}H_{12}O_8$, a phenolic acid (*P. griseo-fulvum*).

(4) *Complex aliphatic compounds* : Spiculisporic acid, $C_{17}H_{28}O_6$, the γ-lactone of γ-hydroxy-βδ-dicarboxypentadecoic acid (*P. spiculisporum, P. minioluteum, P. crateriforme*) ; γ-ketopentadecoic acid (*P. spiculisporum*) ; minioluteic acid, $C_{16}H_{26}O_7$, the γ-lactone of αβ-dihydroxy-β γ-dicarboxy-*n*-tetradecanoic acid (*P. minioluteum*) ; penicillic acid $C_8H_{10}O_4$, γ-keto-β-methoxy-δ-methylene- Δ^{α}-hexenoic acid (*P. puberulum, P. cyclopium*).

(5) *Fats and Sterols* : Most moulds synthesize fats and sterols. The fat of *A. Sydowi* contains phospholipin, palmitic, stearic, tetracosoic, oleic, linoleic and more highly unsaturated acids and ergosterol. Ergosterol palmitate (*P. brevicompactum*).

(6) *Polysaccharides* : Mould starch (*A. niger, fumigatus, oryzæ, glaucus, P. variabile, etc.*) ; mycodextran (*P. expansum, A. niger*) ; glycogen (white species of *Aspergillus*). These all give glucose on hydrolysis. Mycogalactan (*P. expansum, A. niger*) ; varianose (*P. varians*) containing glucose, galactose, idose or altrose units ; mannocarolose (*P. Charlesii*), mannose units ; galactocarolose (*P. Charlesii*) galactofuranose units—here the structure closely resembles that of the acids produced by the same organism, suggesting that the polysaccharide is an intermediate in their formation ; luteic acid containing 2 glucose to 1 malonic acid units (*P. luteum*), etc.

(7) *Protein* is formed by moulds in general ; alkali-soluble protein (e.g., *P. chrysogenum*) glucosamine-containing (*A. fischeri*).

(8) *Enzymes*, e.g., takadiastase, protease, urease, etc.

(9) *Organo-metallic compounds* : *P. brevicaule* growing on media containing As_2O_3 gives trimethylarsine, on ethylarsinate gives

ethyldimethylarsine, on diethylarsinic acid gives diethylmethylarsine, on propylarsinate gives propyldimethylarsine, on Selenium compounds gives dimethylselenide, on diethyldisulphide gives ethyl mercaptan + methylethylsulphide, on dipropylsulphide gives propyl mercaptan + methylpropylsulphide.

(10) *Other substances of unknown constitution :*

(*a*) Terrein, $C_8H_{10}O_3$, a hydroxycyclopentanone (*A. terreus*).

(*b*) Palitantin, $C_{14}H_{22}O_4$, an unsaturated hydroxyaldehyde (*P. palitans*).

(*c*) Glauconic acid, $C_{18}H_{20}O_7$, and glaucanic acid $C_{18}H_{20}O_6$ (*P. glaucum*).

(*d*) Byssochlamic acid $C_{18}H_{20}O_6$, a dianhydride of a tetrabasic acid (*Byssochlamys fulva*).

(*e*) Chlorinated compounds $C_{16}H_{10}O_7Cl_2$ and $C_{17}H_{12}O_7Cl_2$ (*A. terreus*).

(*f*) Nitrogenous acid $C_{22}H_{28}O_5N_2$ (*P. griseo-fulvum*).

(*g*) Antibacterial substance, penicillin (*P. notatum*).

Prof. T. Chrzaszcz and Dr. K. Leonhard (Poznań) : *Formation of Citric Acid from Lactic Acid.*

When the mould *Botrytis cinerea* was grown for 10 days at 22° on a medium containing 2·5 per cent. calcium lactate and 1 per cent. $CaCO_3$, it was found that 50·7 per cent. of the lactate was utilized, of which 8·3 per cent and 15 per cent. were converted to citric and oxalic acids respectively, and the remaining 27·4 per cent. to CO_2 and small amounts of malic, fumaric, acetic, propionic and pyruvic acids and acetaldehyde. Similar results are obtained using 5 per cent. calcium lactate + 1 per cent. $CaCO_3$ and with 5 per cent. ammonium lactate + 3 per cent. $CaCO_3$ grown for 18 and 21 days respectively. Using ammonium lactate without $CaCO_3$, the consumption of lactate was greater (58·3 per cent.), of which 1·4 per cent. was recovered as volatile acids and citric acid, 56·9 per cent. as CO_2, but oxalic acid was not obtained.

When the mould was grown on a medium containing 2·7 per cent. ethyl alcohol + 4 per cent. $CaCO_3$ for 18 days, 90·7 per cent. of the alcohol was utilized, of which 5·947 per cent. was recovered as citric acid, 0·036 per cent. as malic acid, 0·131 per cent. as formic acid, 0·84 per cent. as acetic acid, 0·795 per cent. as oxalic acid and 82·95 per cent. as CO_2.

Lactic acid under the action of the moulds is, therefore, first converted into pyruvic acid which is decarboxylated to acetaldehyde. This is either oxidized into acetic acid or with a molecule of lactic acid undergoes dismutation into propionic and acetic acids. The acetic acid formed is converted via succinic, fumaric and malic into citric acid, or is oxidized via glycollic, oxalic and formic acids

into CO_2 and water. In older cultures, the citric acid formed possibly also undergoes decomposition.

Prof. F. Challenger (Leeds) : *The Methylation of Compounds of Arsenic, Selenium and Sulphur by* Penicillium brevicaule.

Bread cultures of *Penicillium brevicaule* convert inorganic compounds of arsenic and selenium to trimethylarsine and dimethyl selenide (*J. Chem. Soc.*, 1933, 95 ; 1934, 68). Tellurium compounds give a volatile product, doubtless dimethyl telluride, but this has not yet been identified. On administration of selenites and tellurites to animals, elimination of volatile compounds occurs in the breath. In view of the well-known methylating action of animal tissues, from the odour and from analogy with " selenium moulds " these compounds are presumably dimethyl selenide and telluride. Patients receiving inorganic arsenic by the mouth have recently been shown (Pleschtizer, *B. Chem. Abs.* (A), 1935, 1022) to eliminate a volatile unidentified arsenic compound in the breath.

Alkylarsonic acids ($RAsO_3H_2$) and dialkylarsonic acids ($R_2AsO{\cdot}OH$) are converted by the mould to methylated alkylarsines $RAs(CH_3)_2$ and R_2AsCH_3 (*J. Chem. Soc.*, 1935, 396 ; 1936, 264). The wider aspects of biological methylation are described in *Chemistry and Industry*, 1935, 657, and in *Biochem. J.*, 1935, 1757, where further experimental work and possible mechanisms are discussed. Many common inorganic and organic sulphur compounds resist methylation by the mould, but methyl-, ethyl- and *n*-propyl disulphides (RS-SR) are converted to RSH and $RSCH_3$ (*Chem. and Ind.*, 1936, Feb. 21). The mercaptans are absorbed in mercuric cyanide and characterized as the mercaptides, $Hg(SR)_2$. Unchanged disulphides and the methylated monosulphides $RSCH_3$ pass on and are absorbed in aqueous mercuric chloride. The resulting deposits with cold sodium hydroxide liberate pure methyl sulphide, methyl ethyl sulphide and methyl *n*-propyl sulphide respectively. From these the mercurichlorides, m.p. and mixed m.p.s 158°, 127-8° and 163·5-165° are obtained. The unchanged disulphides undergo fission in presence of the mercuric chloride, giving e.g., $C_2H_5SHgCl.HgCl_2$ and $C_3H_7SHgCl.HgCl_2$, which yield no volatile product with sodium hydroxide.

Absorption of the methyl ethyl sulphide in alcoholic benzyl chloride and treatment with sodium picrate give benzylmethylethylsulphonium picrate, m.p. and mixed m.p. 100·5-101° with a synthetic specimen.

The methyl *n*-propyl sulphide has also been characterized as benzylmethyl-*n*-propylsulphonium picrate, m.p. and mixed m.p. 95-95·5° ; the dimethyl sulphide also as benzyldimethylsulphonium picrate m.p. and mixed m.p. 133·5-134°.

The evolution of dimethyl sulphide by certain seaweeds established by Haas (*Biochem. J.*, 1935, 1297) may be noted.

Dr. T. K. Walker (Manchester): *The Formation of Citric Acid from Glucose by* Aspergillus niger *in the Presence of Iodoacetic Acid.*

Six physiological strains of *Aspergillus niger* which were known to produce citric and oxalic acids from glucose under aerobic conditions, and to give rise to ethyl alcohol when placed in contact with glucose solutions under anaerobic conditions, were allowed to remain in contact with glucose solutions (10 per cent.) in the presence of different concentrations of sodium iodoacetate under both aerobic and anaerobic conditions.

The iodoacetate at a concentration of M/500 prevented the formation of ethyl alcohol under anaerobic conditions, but exercised little or no effect on the yields of citric acid formed under aerobic conditions. Formation of citric acid was not suppressed entirely until the iodoacetate was employed at concentrations greater than M/400.

Since iodoacetic acid prevents the conversion of triosephosphoric acid to pyruvic acid, it follows from these results that the production of citric acid from glucose by mould fungi does not depend upon a preliminary process of alcoholic fermentation, as formerly suggested by some workers. This conclusion receives independent support from the work of Bennet-Clark with strains of *Penicillium* in the presence of iodoacetic acid (private communication), and it has been confirmed by the fact that yields of citric acid from glucose much in excess of the highest yield (71 per cent.) theoretically obtainable *via* ethyl alcohol have been reported by a number of investigators recently; thus Raistrick and collaborators have recorded yields up to 87 per cent. of the weight of the sugar consumed, and Butkewitsch and also Wells, Moyer and May have reported yields up to 90 per cent.

Dr. O. E. May (Washington, D.C.) [Communicated by Dr. K. S. Markley (Washington, D.C.)]: *The Production of Lactic Acid by the Lower Fungi.*

The fact that certain genera of the lower fungi produce lactic acid from glucose in good yields is not generally appreciated. This situation undoubtedly has arisen because the more commonly occurring moulds, such as the Aspergilli and the Penicillia, have been more intensively investigated from the biochemical standpoint than other genera. Lactic acid production seems to be confined to the Mucorales. Certain species of *Rhizopus* in particular form large quantities of lactic acid, together with smaller quantities of fumaric, succinic, and possibly malic acids, and ethyl alcohol. The exact relationships which might exist between the mycological breakdown of glucose to lactic acid, and certain of the bacterial lactic fermentations appear to be of considerable interest.

Dr. M. J. Johnson, Dr. J. Berger and **Prof. W. H. Peterson** (Madison, U.S.A). [Communicated by Dr. A. G. Norman (Rothamsted)]: *Some Components of the Mould Proteolytic Complex.*

The peptidase system of moulds consists of a large number of components. Previous work on *A. parasiticus* established the existence of at least five peptidases, and recent work has corroborated and extended these findings. About thirty moulds, including many representatives of the Aspergilli, Penicillia, and other groups, have been investigated. The activity of aqueous extracts of the mycelium of each species toward various substrates was determined. The substrates used were leucyldiglycine, triglycine, leucylglycine, diglycine, chloroacetyltyrosine and gelatin. Large variations occurred in the peptidase activity of different moulds toward a given substrate even in the case of closely-related species. Likewise, the ratio between activities towards any two substrates varied greatly with different species. For instance, the ratio of leucyldiglycine activity to triglycine activity varied from 0·6 in *A. niger* to 74 in. *P. terrestre.* It is apparent from the observed variations that at least five peptidases must be present.

The effect of 12 different organic inhibitors on the hydrolysis of six different substrates by the peptidases of *A. parasiticus* has also been studied. Hydrolysis of no two of the substrates exhibited similar behaviour toward the various inhibitors. This work is further indication of the multiplicity of mould peptidases.

***Prof. N. N. Iwanoff, Dr. V. E. Iwanova** and **Dr. N. N. Larionova** (Leningrad): *The Nitrogen Nutrient of Mould Fungi.*

(1) The nitrogen nutrient of *Aspergillus niger* was studied to determine the synthetic organic compounds of nitrogen, such as biuret, methyl- and dimethyl-urea, which cannot be assimilated, even by fungi under conditions of nitrogen starvation. An observation of the growth of fungi on these compounds indicated that, depending upon the conditions, they are partially decomposed by the action of chemical (*p*H of the medium) and physical (temperature) factors.

(2) Compounds containing methylated nitrogen, as, for example, betaine, and also heterocyclic nitrogen compounds like nicotine, quinine and pyridine, may be utilized by the fungi in a condition of nitrogen starvation, since for these products, which are found in nature, the fungus produces corresponding ferments.

(3) The last group of compounds to be studied was those of hydrocyanic acid, which, as the sole source of nitrogen, increased the growth of fungi in 100·0 c.c. of nutrient solution to 1·2 gms. dry weight. The addition of sulphur to the nutrient medium has an exceeding high stimulatory action toward the assimilation of hydrocyanic acid by the fungus.

Prof. W. H. Peterson (Madison, U.S.A.). [Communicated by Dr. A. G. Norman (Rothamsted)]: *Studies on the Composition of Mould Mycelium.*

By the use of a specially-designed sterilizer incubator containing shallow aluminium pans, large quantities of mould mycelium have been grown for chemical and biological studies. The dry weight of mycelium obtained has been about one quarter of the glucose fermented. The conditions providing for the highest yield of mycelium are different from those giving the maximum amount of fermentation products.

The chief constituent groups investigated were the carbohydrates, lipids, and nitrogenous substances, while, in addition, the vitamin content and nutritive value of the whole mycelium has been studied.

The main carbohydrate fraction soluble in alkali gave an intense blue colour with iodine, and was difficult to obtain nitrogen free. Glucose only was given on hydrolysis. The structural material of the tissue appeared to be a carbohydrate very resistant to hot strong alkalis, yielding glucose, glucosamine, and acetic acid on hydrolysis. Pentosans, cellulose, uronic acids and glycogen were absent.

Simple lipids constituted about 80 per cent. and phospho-lipids about 20 per cent. of the total lipid. Palmitic, stearic, *n*-tetracosanic, oleic and linoleic acids have been isolated together with ergosterol and glycerol. A survey of the sterol content of 65 species gave results from 0·03–2·23 per cent. ergosterol on the dry weight, the highest figure being obtained from a single spore culture of *A. fisheri*.

Only about 55 per cent. of the nitrogen was found as true protein, and this appeared to be in association with carbohydrate. From the water soluble fraction leucine and *iso*leucine were obtained free. Other amino-acids were obtained after brief autolysis.

Mould tissue did not provide a complete protein ration for rats, even though 70 per cent. of the nitrogen was digested. The deficiency, the nature of which is not yet known, could be corrected by the addition of small quantities of other proteins. When fed at a level of 10–20 per cent. mould mycelium supplied sufficient Vitamin B complex to provide good growth. The B_1, B_2 and B_4 components were present in moderate amounts, the mould tissue being about half as rich in Vitamin B_1 as yeast grown under similar conditions.

Prof. M. Schoen (Paris).

Le fait que *l'Aspergillus niger* produit de l'acide citrique aux dépens de l'acide acétique prouve simplement que ce dernier subit une oxydation. Ce qui fournit, en fin de compte, l'acide citrique ce serait précisément *l'acide glycolique*. Du reste, on sait depuis

l'acide citrique sous l'influence de l'action de certaines moisissures (Mazé). L'alcool éthylique, pas plus que l'acide acétique, n'est pas cependant un corps intermédiaire dans la fermentation citrique. Celle-ci doit-être envisagée comme le résultat d'une *respiration déviée*. Or, la respiration ne se distingue des processus de fermentation proprement dite que par la manière dont l'hydrogène, véritable produit intermédiaire, est stabilisé en même temps que son compagnon obligatoire, l'aldéhyde acétique. C'est donc dans les produits de l'oxydation de ce dernier qu'il faut chercher les prédecesseurs de l'acide citrique. L'acide glycolique remplit bien ce rôle.

Le fait bien connu que *l'Aspergillus niger* fournit de l'acide carbonique confirme bien ce qui vient d'être dit. M. Chrzasczcz a tort lorsqu'il traite cette conception, comme toutes les autres similaires, de fantaisistes. Ce qu'on avance ici n'est que "*l'expression prochaine*" des *faits expérimentaux*, connus jusqu'a aujourd'hui et l'on est tout prêt d'abandonner cette conception, si des faits nouveaux venaient de l'infirmer.

(Voir pour les détails : *Annales de l'Institut Pasteur*, T.47, 1931, p. 690 et *C.R. du Xè Congrès de Chimie Industrielle*, 1930. — Les bases scientifiques de quelques nouvelles industries de fermentation.)

Prof. T. Chrzaszcz (Posnañ).

First of all, in answering Dr. Schoen, I wish to state that I am referring only to actually found products of the citric acid fermentation. I cannot discuss theories or assumptions which look interesting while written on paper, but are not proved in actual laboratory or commercial investigations.

I wish to emphasize again that citric acid is formed from ethyl alcohol, acetic acid, malic acid and, as we found recently, also from lactic acid. But we never found aconitic acid or quinic acid. The problem is still unsolved, whether for the formation of citric acid the sugar molecule has to be transformed into ethyl alcohol and acetic acid or whether citric acid may be formed directly in a simpler way.

Neither our own investigations nor the experiments of other workers have added till now anything to the last-mentioned problem.

JOINT DISCUSSION *with* Section 4 *on* YEAST METABOLISM

Chairman : Sir Arthur Harden (London).

Prof. A. Fernbach (Paris), **Joint Opener.**

In this short paper, under the above title, it is intended to deal exclusively with the influence of different factors upon the growth and development of the yeast cell.

Prof. R. H. Hopkins contributed to this question last year, in Brussels, at the IVth International Congress for Agricultural Industries, in an important paper to which special reference must be made. After discussing the methods that may be applied towards a solution of the problem, Prof. Hopkins refers to the investigations of Thorne concerning the nitrogen metabolism of yeast,[1] and the rather poor crops obtained by introducing different nitrogenous compounds into an artificial medium, and concludes that the influence of the following factors should be studied: (1) *p*H of medium, (2) concentration of nitrogenous nutrients, (3) aeration, (4) the presence or formation of inhibiting substances, (5) the nature of " bios " or " growth-promoting factors." These headings are used in the following sections of the paper, the conclusions of which are briefly reported hereafter.

Reference is made to the work of Taxner,[2] who, on buffering an artificial medium with lactic acid-sodium lactate, found that an English brewery yeast grew best on asparagine as nitrogen source with an initial *p*H of 3.5-3.9. We may fully endorse one of the remarks of Prof. Hopkins by saying that the knowledge acquired by studying the processes taking place in the surroundings of the cell is only a blurred and often incorrect image of what is going on in its protoplasm. Prof. Hopkins insists on the value of ammonia nitrogen as nutrient.

Prof. Hopkins refers to the important work of Hartelius[3] on " The growth of yeast in synthetic media and the factors produced by yeast which limit this growth." It is shown that the main limiting factor is alcohol, the effect of which is closely related to the *p*H of the medium and the increase of acidity. The paper of Klem[4] is then mentioned, and in the fifth section (" Bios ") reference is made to the work of Nielsen and Hartelius[5] on the separation of Nielsen's growth-promoting factor into two substances.

Amongst the factors influencing the growth of yeast, great importance must be attributed to *metals* and their salts; these generally follow the laws of antiseptic action, inhibition commencing with a relatively high amount, while very small amounts are favourable. Many workers have studied the influence of *copper*, owing to its frequent use in brewing utensils (vats, coils, etc.). The action of copper is the object of an extensive study by M. H. Van Laer.[6] Guillemet[7] reached conclusions formulated many years ago by Mann, cited by Van Laer, who mentions also an interesting paper published in 1909 by A. C. Chapman.

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[1] *Journ. Inst. Brew.*, 1933, **39**, 597, 608.
[2] *Ibid.*, 1935, **41**, 27.
[3] *C. R. Lab. Carlsberg*, 1934, **20**, No. 7.
[4] *Hvalradets Skrifter*, 1933, No. 7, 55.
[5] *C. R. Lab. Carlsberg*, 1932, **19**, No. 8.
[6] *Ann. Zymol.*, 1934, Ser. *II*, **1**, 287; *Ann. Brass. Dist.*, **32**, 225 & 241.
[7] *C. R. Soc. Biol.*, 1934, **116**, 1404.

The effect of ***aluminium*** has been studied by De Wilde[8] and by Trautwein,[9] who found that a concentration of 0,01*M* is lethal to yeast, and that one-tenth of this concentration stops multiplication. This, however, is in mineral solutions; in beer wort no noxious effect is seen because phosphates transform aluminium into insoluble compounds.

The influence of ***thallium*** has been studied by D. W. Richards[10], [11], who stated that this metal is sometimes an impurity in asparagine, which is frequently employed as a source of nitrogen for yeast. At a concentration of 1/1000 mg. per c.c., thallium acts as a "bios," increasing the growth of yeast by 80 per cent., while a concentration of this extremely toxic metal of 10 mg. per c.c. stops development completely.

The part played by ***magnesium***, especially in agriculture, has been studied in an important paper by M. Javillier,[12] who cites, amongst others, the work of Lasnitzki and Szörenyi,[13] and of Rabinowitz-Sereni.[14]

Growth-promoting factor, Aeration and Respiration of Yeast.

Although our knowledge of "bios" is limited, some progress has been made by N. Nielsen.[15] Reference must also be made to a paper of E. Janssens.[16]

Aeration has always been considered an important factor in the multiplication of yeast, and is widely used in yeast manufacture; in Effront's process, by aerating the wort and by maintaining the composition of the medium constant by the addition of fresh wort, the formation of alcohol has been practically excluded with a corresponding increase in the yeast yield.

The older theory, however, that there is a close relation between respiration and aeration has had to be abandoned after the impulse initiated through the discovery of glutathione, and the study of compounds found in the yeast cell, which act as respiration factors in systems endowed with a Redox (reduction-oxidation) potential. Such are two respiratory pigments, "yellow ferment" and "cytochrome," the first being a derivative of lactoflavin (vitamin B_2), and the second a ferrohæmin reversibly transformable into ferrihæmin.

Prof. R. H. Hopkins (Birmingham), **Joint Opener** : *Specificity in Fermentation.*

Brewers' and most yeasts exhibit absolute specificity towards the hexoses *d*-glucose, *d*-mannose, *d*-fructose, and, in certain circum-

[8] *Ann. Zymol.*, 1934, Ser. II, 1, 365.
[9] *Z. Ges. Brauw.*, 1934. *J. Soc. Chem. Ind.*, 53, 903 B.
[10] *Arch. f. Protistenk*, 1933, 87, 263.
[11] *J. Biol. Chem.*, 1933, 96, 405.
[12] *Bull. Soc. Chim. Biol.*, 1936, 18, 255.
[13] *Biochem. Journ.*, 1934, 28, 1678.
[14] *Boll. Staz. Patol. Veget.*, 1933, 13, 203.
[15] *C. R. Lab. Carlsberg*, 1934, 20, *No.* 1.
[16] *Arch. Intern. Physiol.*, 1935, 40, 257; *Ann Ferm.*, 1, 117.

stances, *d*-galactose Fermentable di- and tri-saccharides, trioses, hexose derivatives, etc., are fermented by virtue of either preliminary fission or synthesis into one of the above hexoses. There is, however, some evidence that, e.g., maltose may be directly fermented. Yeasts also exhibit what appears to be relative specificity, or, at least, selective preferences among these hexoses and their α and β forms. In fermenting mixtures, glucose is preferred to fructose, and glucose or fructose to mannose. There is no evidence that diffusion is a controlling factor, although this possibility remains. Neither sugar is transformed into the other. Selection is in accordance with the theory and mathematical formulation of the kinetics of enzyme action of Michaelis and Menten. When two substrates compete for the same enzyme, the relative rates of the reactions promoted are expressed by the formula :

$$\frac{V' K_m}{V K'_m} = \frac{\ln y_o - \ln y}{\ln Z_o - \ln Z}$$

where K'_m and K_m respectively are the Michaelis constants,
V' and V are the maximum velocities attainable,
y_o and z_o are initial concentrations of substrates,
y and z are the concentrations remaining at the time of measurement, respectively.

Measurement of y_o z_o y z in a fermenting solution of glucose and fructose leads to values of the above fraction ($K_{G/F}$) which remain constant within the limits of experimental error. Thus an English brewers' yeast gave the value of 2.10, varying between 2.07 and 2.13, except at the extremes of the fermentation.

The fermentations of the sugars in separate solutions also conform to the Michaelis theory. On plotting the reciprocals of velocity of fermentation and of sugar concentration against one another, a straight line is obtained with glucose, fructose, mannose and galactose, as required by the theory. From the graphs can be read off the values of V (maximum velocity) and of $\frac{K_m}{V}$ (slope of graph) whence the value of K_m is obtained. V is approximately the same for glucose, fructose, mannose (and their components) when brewers' yeast is employed, so that the expression $V' K_m / V \cdot K'_m$ simplifies to K_m / K'_m, the inverse ratio of the Michaelis constants. Thus for glucose, fructose and mannose the values 0.192, 0.404 and 0.86 (expressed as concentration per cent.) were respectively obtained in agreement with $K_{G/F} = 2.10 \left(= \frac{0.404}{0.192}\right)$ for mixtures of glucose and fructose, and for glucose and mannose, $\frac{0{\cdot}86}{0{\cdot}192} = 4.5$, whereas direct determination of $K_{G/m}$ for a similar yeast gave the value 3.8.

The yeast enzyme which first combines with the substrate sugar seems to possess a greater affinity for that substrate, e.g., for glucose than for fructose. This may in its turn depend on the proportion present in these sugars at equilibrium of the specifically fermentable component. In single sugars in solution at equilibrium α is preferred to β glucose, α to β mannose, another component of fructose to β(2,6) fructose. Whilst brewers' yeast prefers α to β glucose, nevertheless, the two sugars are fermented at equal rates in separate solutions even when the rates are much greater than that of mutarotation of α or β glucose to equilibrium. From this it is clear that the yeast is not specific for the α form only, but ferments the α and β forms at different rates. Again, at very low concentrations, under suitable conditions, α glucose is fermented more rapidly than β glucose; the Michaelis constants have been found to be, α glucose 0.178, β glucose 0.212. It is not clear whether the glucopyranose forms themselves are directly attacked, or whether a common intermediate form into which they change on passing into solution is attacked. There is some evidence for the latter view. In accordance with either hypothesis it has been found that, in separate solutions at suitable low concentrations, fructose and mannose at equilibrium are respectively fermented faster than the β forms of these sugars.

***Prof. P. Lindner** (Berlin). *Die Verfettung der Hefe.*

Sie ist mikroskopisch leicht festzustellen, daher didaktisch besonders wertvoll. Sie erscheint in Form von Granulationen, die im Plasma verteilt sind oder in massiven Kugeln von starkem Lichtglanz. Die Fettnatur der Granula blieb lange Zeit unerkannt. Sie trat aber deutlich hervor beim Eintrocknen und Wiederanfeuchten der Zelle : Sie verschmolzen dann zu einer oder mehreren massiven Kugeln. Auf festen Nährböden gewachsene Hefenkolonien sind in der Oberflächenschicht besonders stark verfettet. Das deutete hin auf eine Assimilation von Alkohol unter Mitwirkung des Luftsauerstoffs. Eine ähnliche Fettbildung tritt ein, wenn man Alkoholdämpfe zu dickbreiiger Hefe treten lässt, oder wenn man Hefe in Tröpfchenkulturen züchtet, in denen ja reichlich Luft vorhanden.

Eine überaus wichtige Beobachtung war, dass stark verfettete Zellen, namentlich mit Granulationen bei Zugabe frischer Nährlösung nicht mehr aussprossen. Die Gründe für dieses Verhalten sind uns bisher rätselhaft geblieben, zumal verfettete Zellen durchaus noch lebenskräftig aussehen und nicht plasmolysiert.

Die Züchtung des *Endomyces vernalis* versagt, wenn wir den Pilz erst völlig verfetten lassen. Dann geht keine Zelle an. Die Entnahme der Pilzhaut zu neuer Impfung muss schon geschehen, wenn die Zellen noch die Eiweissgeneration darstellen.

Hähn und Kintoff haben gezeigt, dass der Fettpilz keinen Alkohol aus Zucker erzeugt, wohl aber bei Darbietung von Alkohol Fett

liefert, ebenso wie aus Azetaldehyd, Brenztraubensäure, Milchsäure, Aldol und Glyzerin, die einer Eiweissgeneration der Pilzhaut unterschichtet wurden. Ausser Fett wurde reichlich Kohlensäure gebildet aus der Glukose, 30-35 prozent.

Bei der Assimilation des Alkohols zu Fett entsteht nur eine ganz geringe Menge Kohlensäure, dagegen reichlich Wasser. Das erklärt die schnellere Fettbildung, denn hier kann der Sauerstoff ungehindert zu den Plasmagebilden, den Chondriokonten, an denen sich das Fett bilden soll, wie Guillermond annimmt.

Die über den Kühler gelaufene Bierwürze enthält soviel Sauerstoff gelöst, dass die gut gelüftete Anstellhefe die 4-5 fache Fettmenge in der neuentstandenen Hefeernte aufweist. In einer ungelüfteten Würze entsteht kein Fett, wie Stockhausen und F. Windisch gezeigt; auch in einer Pasteurschen und Wildier'schen Lösung kann die Hefe kein Fett bilden, wenn die Lösung kurz vor der Impfung erhitzt worden war. Sie wird in diesem Falle auch bei Spuren einer Aussaat wachsen können. Eine einzelne Kahmhefenzelle, die Fett überhaupt nicht bildet, daher nicht verfetten kann, wird auch bei reichlich Sauerstoff ohne " Bios " wachsen.

Liebig und Wildiers hatten Miserfolg, weil ihre Lösungen offenbar längere Zeit gestanden und Sauerstoff aufgenommen hatten. Ihre Aussaatzellen verfetteten daher zu schnell.

Da immer noch das Biosproblem bearbeitet wird, mag hier erwähnt werden, dass Tanner nach Studium von 150 Biosarbeiten die Verfettungstheorie als diejenige bezeichnet hat, welche die Kontroversen besser erkläre als die anderen.

Wer sich mit dem Fettproblem näher befassen will, muss sich unbedingt mit der Tröpfchenkultur vertraut machen und mit den kleinen Tricks, die auch dabei nicht entbehrt werden können.

Sie ist zwar in Gärungsbetrieben und in der Milchwirtschaft zu grösster Verbreitung gelangt, aber hier zumeist zum Zwecke der Betriebskontrolle, nicht zum wissenschaftlichem Studium.

Wer mikrophotographische Beweise für das oben Gesagte wünscht, möge in Lindner: "*Atlas der mikroskopischen Grundlagen der Gärungskunde*" die Bilder 518,1388-92, 716,1203-1209, 487-489, 889-890,1223 und 1262 nachsehen. 3 Auflage, Paul Parey, Berlin, SW 11.

Dr. L. R. Bishop (Rothamsted, England): *Nitrogen Metabolism of Yeast.*

Interest in brewing is concerned with many aspects of yeast metabolism. Considerable interest and discussion centre round the nitrogen nutrition of yeast of which studies have been made by two general methods—the synthetic and analytic. The primary difficulty in the synthetic method of approach is the need for concentrated supplies of the yeast accessory growth factor—bios. The existence and nature of bios has been long under discussion and doubt, but fair agreement has now been reached on its

nature by workers in different countries. The present studies reconcile some apparent contradictions in this work. The bios requirements of yeast and the supplies in brewers' wort have been compared.

The primary difficulty in the analytic studies of yeast nitrogen nutrition has been the need for methods of isolation and analysis of the nitrogen compounds of wort. This has been overcome and the course of yeast nitrogen assimilation is described.

Dr. Niels Nielsen (Copenhagen): *The Assimilation of Amino-acids by Yeast.*

The ability of yeast to assimilate different nitrogen compounds is often examined by cultivating yeast in a synthetic nutrient solution, with the nitrogen compound whose assimilability is to be examined as the only source of nitrogen. Growth of the yeast is taken as evidence that the compound is assimilable. This method, however, presents one difficulty. The yeast can neither grow nor assimilate nitrogen if the solution does not contain growth substance (" Bios "); even when easily assimilable nitrogen is contained in the nutrient solution. Sufficient " growth substance " must, therefore, be added to the nutrient solution (an addition of 0.5 per cent. of wort is sufficient). This will, of course, supply some nitrogen, but it is possible to make an appropriate correction with accuracy.

The yeast may increase its dry matter content as much as three-fold without assimilating nitrogen, and it will then contain proportionately less nitrogen. Determining the growth of yeast may, therefore, not indicate assimilation of nitrogen. The assimilation of nitrogen ought to be measured directly by determining the amount of nitrogen in the nutrient solution.

Applying this method to *Saccharomyces cervisiæ* we have examined its ability to assimilate 30 amino-acids. It was found that only α amino-acids were easily assimilated, β, γ, and ε amino-acids were not assimilated. δ amino-acids were assimilated very slowly. Probably in the last case a re-arrangement of the amino-acid occurs. The α amino-acids are only assimilated if they do not contain too long carbon chains; thus amino-adipic-acid, diamino-adipic acid, diamino-pimelic-acid and lysine (α, ε diamino-capronic-acid) were not assimilated.

In the case of histidine only one-third of the nitrogen is assimilated, and with tryptophan only one-half. Thus it appears that it is the amino-group of the alanine side chain which is utilized. This suggests that the amino-acids are split outside the yeast cell. The assimilable amino-acids can also be assimilated in a few hours, provided a solution of them is shaken with large amounts of yeast. Assimilation only takes place when sugar is contained in the solution and when fermentation occurs.

Prof. E. A. Pribram (Chicago): *The Influence of Potassium and Calcium Ions on the Micrometabolism of Yeast Cells of Plant and Animal Origin.*

Potassium chloride and calcium chloride have opposite effects on the esterification of glucose by yeast cells of plant or of animal origin (beer yeast, milk yeast, yeast from thrush). Potassium chloride decreases, while calcium chloride increases the combining power of beer yeast, *Saccharomyces cerevisiæ* (Frohberg), for glucose. Potassium chloride increases and calcium chloride decreases the combining power of animal and human yeasts for glucose: *Torulopsis "kephir"* (Beijerinck); *Cryptococcus hominis* (Busse), from thrush. The opposite effect is evidently due to the difference in the potassium and calcium contents of plant and animal yeasts. The ratio between the two cations is as follows: K:Ca = 10:1 (up to 28:1) in the yeast of plant origin (beer yeast), and 9:14 or 1:1.55 in the yeast of animal origin (milk yeast). The ion predominating in the yeast acts as a catabolic catalyst, if added to the fluid in which the cells are suspended (saline solution), whereas the complementary ion has an anabolic action.

Suspensions of 1,000 yeast cells per c.mm. in isotonic saline solution have been used. Two mgm. of dextrose per c.mm. and 0.02 cc. of $N/10$ solution of the salts were added. All cultures were three days old, grown on dextrose agar.

DISCUSSION *on* INTERMEDIATE CARBOHYDRATE METABOLISM OF MICRO-ORGANISMS

Chairman : Sir Arthur Harden (London).

Chairman's *Introductory Remarks.*

Buchner, by the preparation from yeast of a cell-free extract which had the power of fermenting sugar, not only settled the question at issue between Liebig and Pasteur as to the nature of fermentation, but provided a valuable material for the study of the intermediate stages in the fermentative change of sugar into alcohol and carbon dioxide. The disorganization of the cell had modified the conditions in which the change was effected and had rendered it susceptible to the influence of many substances which were without perceptible action on the living cell.

By the study of materials of this kind the sum of knowledge has been acquired which is to form the subject of discussion to-day. In addition to the effects of drying and disintegration, those of chemical inhibitants, fluoride, iodoacetic acid, toluene, etc., have also been utilized, as has the use of dialysed extracts and partially purified enzymes to gain information about the intermediate stages in the conversion of sugars into the final products of metabolism,

and a very detailed knowledge of these intermediate stages is now available.

One important problem which remains to be solved is to retrace the path which Buchner took, to proceed from the cell-free preparation back to the living cell. What is the balance of reactions which leads to the products characteristic of the living cell, smoothly and continuously produced without any accompanying accumulation of by-products? The form in which this question is proposed implies the belief, which I hold as a very strong probability, that the reactions in the living cell are essentially of the same kind as those which occur in cell-free preparations, but differently co-ordinated. I am aware that opposing views have been expressed, but I do not think that they are well founded.

Another question of the moment is whether the mode of attack on the sugar molecule which is used by yeast and muscle—and also by some bacteria and higher plants—is the only biological method for the metabolism of this class of compound, or whether an alternative route based on a different series of reactions also exists. The question is one which can only be settled experimentally, but its study is difficult and beset with pitfalls. Even with preparations from a well-studied organism like yeast it is possible to arrange a set of conditions in which many of the criteria of alcoholic fermentation fail to be exhibited and it must be expected that in tissues of varied origin extreme variations in the proportions of the different agents may exist, so that very exhaustive analysis of the phenomena is necessary before conclusions can be drawn.

Prof. A. J. Kluyver (Delft), **Opener.**

The subject of carbohydrate metabolism embraces two main groups of processes, viz., aerobic breakdown or respiration processes and anaerobic breakdown or fermentation processes. For the study of this metabolism micro-organisms offer many advantages. Many organisms are readily accessible as pure cultures and in using these the investigator has the certainty that he is dealing with a uniform biocatalytical material. Moreover microbial carbohydrate metabolism is characterized by its great diversity, thus permitting comparative studies.

It is nowadays generally accepted that as a rule biochemical conversions do not proceed in one step but that they must be considered as the outcome of a shorter or longer chain of primary reactions, and hence the question arises: by which methods can the intermediate products occurring in a given biochemical process be identified?

On surveying the literature it appears that the following methods have found the widest application:

(I) The so-called fixation method ("Abfangmethode"), initiated by *Neuberg*, which consists in adding to the reaction medium a compound with a special affinity

towards one of the presumed intermediate products. The product in question may therefore be fixed, i.e., withdrawn from the normal course of the reaction.

(2) The method of disharmonizing the various components of the catalytical system of the living cell by the preparation of so-called cell-free systems. This procedure should automatically lead to an accumulation of intermediate compounds or their stabilization products. It was this method which led to *Harden and Young's* discovery of the formation of the hexose phosphoric esters in carbohydrate metabolism and which, in later years, has been so successfully applied by *Neuberg* (more or less complete elimination of specific catalysts by dilution of cell-free preparations).

(3) The method of selectively poisoning parts of the catalytic system. The highly important results obtained by *Meyerhof* in recent years are chiefly due to the application of specific poisons like sodium fluoride and iodoacetic acid.

All these methods may lead, under favourable conditions, to the isolation, often in the crystalline state, of special products (or their derivatives) which are not obtained in the normal process. Until recently such an isolation has nearly always been considered as a definite experimental proof of the occurrence of these products as intermediate stages in the process under investigation.

It has, however, to be emphasized that in all cases this conclusion includes several hypothetical elements. When applied to the results of the fixation method it is tacitly assumed that the compound added restricts its activity to the fixing of a normally occurring intermediate product; the possibility of its altering the normal course of the reaction is left out of consideration. In the cases of the disharmonizing or selective poisoning methods it is likewise assumed that only special parts of the catalytic system are removed and that the remaining parts of the system remain wholly unaffected.

The fact that in some cases, as e.g., in that of alcoholic fermentation, biochemistry is nowadays suffering from a surplus of intermediate products, shows conclusively the untenability of these assumptions. The drawing up of a scheme for the internal mechanism of a biochemical process on the basis of isolated " intermediate products " always remains, therefore, an operation of a speculative nature.

It is obvious that under these conditions one can only judge the merits of a proposed scheme by testing its ability to fulfil the requirements imposed by all experimentally well-established facts available.

In the case of the more complex breakdown processes, as are typical for several bacterial fermentations, it can easily be shown

that a determination of the quantitative relations in which the different products occur in the reaction medium can be of decisive importance.

In those cases in which the cells under investigation are characterized by the two main types of carbohydrate breakdown, viz., fermentation and respiration, a study of the inter-relationship of these processes may also yield a valuable clue to the interpretation of their internal mechanism.

Summarizing, it seems advisable to realize that the establishment of the nature of intermediate metabolism will always remain a delicate mental process of co-ordination.

Prof. O. Meyerhof (Heidelberg): *Zusätze zum Gärungsschema.*

Anschliessend an die kürzlich mitgeteilten Schemata der alkoholischen Gärung und des Hauptwegs der fermentativen Milchsäurebildung werden die verschiedenen, hierbei vorkommenden Reaktionsmechanismen besprochen, vor allem ein neuer, zusammen mit K. Lohmann aufgefundener Reaktionstyp; die enzymatische Aldolkondensation durch ein als *Aldolase* bezeichnetes Ferment. Für die Gärung sind 2 derartige Reaktionen von Interesse, die Kondensation von Acetaldehyd mit Dioxyacetonphosphorsäure zu Methyltetrosephosphorsäure und diejenige von Glycerinaldehyd mit Dioxyacetonphosphorsäure zu Fruktose-1-phosphorsäure. Auf Grund der letzteren Reaktion wird auch die Zymohexasereaktion

$$\text{Hexosediphosphat} \rightleftharpoons 2\ \text{Dioxyacetonphosphat}$$

jetzt genauer so formuliert:

$$\text{Hexosediphosphat} \longrightarrow \text{Dioxyacetonphosphat} + \text{Glycerinaldehydphosphat} \rightleftharpoons \text{Dioxyacetonphosphat.}$$

Bei den Umesterungsreaktionen besteht eine Übereinstimmung zwischen dem freien Adenylsäuresystem K. Lohmanns und der Harden-Eulerschen Co-zymase; auch die letztere bildet dabei eine von W. Kiessling isolierte Pyrophosphorsäureverbindung. Dagegen gehen die Oxydoreduktionen der Gärung nur mit Hilfe der Co-zymase, nicht des freien Adenylsäuresystems von statten.

Prof. C. H. Werkman (Ames, U.S.A.): *Bacterial Dissimilation of Glucose.*

Initial phosphorylation of the glucose molecule is of general occurrence. Uptake of phosphorus has been shown with: *Clostridium*, *Aerobacter*, *Citrobacter*, *Lactobacillus*, *Serratia*, *Propionibacterium*, *Bacillus* and *Escherichia*. The hexose phosphate is converted into phosphoglyceric acid apparently through dihydroxyacetone phosphate. α-Glycerol phosphate may be a normal hydrogen donator. It is readily dissimilated to acetic and formic acids.

Re-evaluation of the rôle of methylglyoxal, generally accepted as the key intermediary in bacterial metabolism, is necessitated by the isolation of phosphoglyceric acid from the dissimilation of glucose and hexose diphosphate by a variety of micro-organisms: (from hexose diphosphate + glucose) *Propionibacterium shermanii, P. arabinosum, P. pentosaceum, Lactobacillus pentoaceticus* (heterofermentative), *L. plantarum* (homofermentative), *Escherichia coli, Citrobacter freundii, Aerobacter aerogenes, Bacillus subtilis,* and *Serratia marcescens.*

The method of Vercellone and Neuberg modified to bacterial requirements was employed. Phosphoglyceric acid was isolated in the absence of added acetaldehyde, when pyruvic acid replaced acetaldehyde, or small yields resulted in the absence of toluene.

Phosphoglyceric acid is transformed into pyruvic acid and phosphoric acid. Convertion is slow, but is stimulated by glucose, and creatine or adenylic acid. Pyruvic acid has been isolated as an intermediary with *Propionibacterium, Escherichia, Aerobacter, Lactobacillus, Clostridium* and *Citrobacter.* Reduction to lactic acid is of general occurrence or dehydrogenation of the hydrate may lead to acetic and formic acids, the latter yielding CO_2 and H_2. This hydrogen reduces such acceptors as pyruvic acid, acetylmethylcarbinol, oxygen, acetaldehyde and acetic acid. Production of gaseous hydrogen is suppressed.

An assimilative phase is shown by utilization of CO_2 from carbonate by the propionic acid bacteria, accompanied by a greater formation of oxidized products, especially succinic acid. Reduction of CO_2 occurs through formaldehyde indicated by isolation of the formaldimethone derivative. A non-reducing fermentable carbohydrate is formed. Additional evidence of the rôle of adenylic acid and creatine in bacterial metabolism is required to provide a complete picture of the dissimilation.

Prof. M. Schoen (Paris) : *Les problèmes des spécificités fonctionnelles dans la dégradation des sucres.*

Il est à remarquer que parmi les méthodes qui conduisent à l'isolement de produits intermédiaires, la méthode basée sur le changement de la réaction du milieu s'est montrée très féconde. C'est ainsi qu'on est parvenu, pour la première fois, à Paris, dès 1912, à mettre en évidence la production de l'acide pyruvique, cheville ouvrière de toute la chimie moderne des fermentations.

M. Schoen développe ensuite la conception du rôle des carences dans les phénomènes de fermentation. Les spécificités biochimiques dépendent de l'état et de la nature des agents catalytiques. Toute déficience dans l'état d'activité de ces agents et toute carence dans leur constitution font varier les fonctions cellulaires.

Ces carences peuvent être congénitales, fixées plus ou moins fortement par l'hérédité de l'espèce. Ces carences peuvent être également *imposées volontairement* et, alors, l'on assiste à des

modifications profondes des fonctions chimiques. Le cas de la phosphorylisation, ainsi que celui de la production de l'acide pyruvique, offrent les exemples les plus typiques de cet état de choses. Dans le premier cas, c'est la levure *estropiée*, qui conduit à la production et à l'accumulation des hexosephosphates ; dans le deuxième cas, c'est le déplacement des vitesses relatives des actions diastasiques qui a pour conséquence l'accumulation de l'acide cétonique.

Cette manière de voir trouve un appui très sérieux dans l'action des catalyseurs chimiquement définis où des carences *effectives* ont également pour conséquence les changements complets des proportions relatives des produits formés. (cas de la condensation de CO et H_2.)

(Voir pour les détails : *Annales Institut Pasteur*, T. 47, 1931, p. 690 ; et *Annales des Fermentations*, T. 1, 1935-36, p. 257.)

Miss Marjorie G. Macfarlane (London) : *Phosphorylation and Nucleic Acid Derivatives in Living Yeast.*

At the onset of fermentation induced by the addition of sugar to fresh brewers' yeast, the amount of inorganic orthophosphate present in the trichloroacetic acid extract of the yeast rapidly decreases and remains decreased till the sugar is consumed, when the original level is regained. The decrease is not balanced by an increase in the acid-soluble organic phosphate and frequently coincides with a decrease in the total acid-soluble phosphorus, indicating conversion of phosphoric acid into an acid-insoluble compound. After extraction of 50 per cent. of the total phosphorus present in brewers' yeast by trichloroacetic acid, a further 30 per cent. was obtained in solution by extraction of the residue with water + NaOH at *p*H 9.0, and recovered as a sodium salt containing 16 per cent. P (of which 80 per cent. was labile in 7′ in *N* HCl at 100°), with P : N ratio = 12 : 14. The analysis and properties agree with those of a nucleic acid associated with a labile phosphoric acid. By repeated extraction in 5 per cent. HCl and reprecipitation with alcohol, 50 per cent. of the total phosphorus in this nucleic acid fraction was recovered and identified as a sodium metaphosphate. The nucleic acid-metaphosphoric compound is probably the " Plasminsäure " described by Kossel (1893) and Ascoli (1899) Zeit, physiol. Chem., **28**, 426.

Comparison of similar fractions isolated from (*a*) resting and (*b*) fermenting yeast indicates a significant decrease, absolute and relative, in the nitrogen content of the nucleic acid fraction during fermentation. (See *Biochem. J.* 1936, **30**, 1369).

Dr. F. F. Nord (Berlin) in Erwiederung auf Prof. Meyerhof und Prof. Schoen sprach :

Gelegentlich der Untersuchung der Kinetik der Hefezellgärung unter dem Einfluss verschiedener Rührgeschwindigkeiten hat sich

herausgestellt, dass die Induktion bei der *lebenden* Zellgärung keine Bedeutung besitzt. Diese Beobachtung liess den Schluss zu, dass ein direkter Vergleich der zellfreien und Trockenhefegärung mit der lebenden Zellgärung erzwungen ist.

Unter Heranziehung des Enzymsystems des weitverbreiteten *Fusarium lini* Bolley, dessen Wirkungsweise biochemisch, biophysikalisch-chemisch und stöchiometrisch mit dem Leistungsmechanismus des Fermentsystems der Hefe vergleichbar ist, hat sich dann bei Versuchen, sowohl mit lebenden Zellen, als auch bei Anwendung von Trockenpräparaten ergeben, dass, der Vergärung von Kohlehydraten (sowohl Hexosen als auch Pentosen) eine Veresterung von freiem anorganischem Phosphat *nicht* voranzugehen braucht, sondern, dass die Auflockerung des Zuckermoleküls wahrscheinlich durch eine Deformierung der Bindungen der Substratmoleküle zustande kommt. Die Veresterung *kann* aber im späteren Verlauf der Reaktion eintreten und trotz der Phosphatase-Wirkung festgehalten werden. Beweise : (1) Der Schwund an anorganischem P beginnt durchschnittlich 2–5 Tage *nach* dem Einsetzen der Gärung und *nach* dem Fassen des sich entbindenden CO_2. (2) Im Gegensatz zur d-Arabinose, wird d-Xylose überhaupt nicht phosphoryliert. (3) Züchtet man den Erreger auf mit Wasser gequollenem Leinsamen in Abwesenheit von anorg. P, so hat es sich bei einzelnen Kohlenhydraten gezeigt, dass die Gärleistung des Systems wesentlich herabgesetzt wird, um sich später, nach erfolgter Nachgabe, von anorg. P, bis zur normalen CO_2-Tagesleistung zu erhöhen, *ohne*, dass auch nur eine Spur einer nennbaren Phosphorylierung festzustellen gewesen wäre. Dagegen ging mit der Regeneration der Gärfähigkeit eine sichtbare *morphologische* Veränderung der Zellen vor sich, die darauf schliessen lässt, dass der P. für den Zellneubau bzw. die Pufferung der Zelle von Bedeutung ist.

Diese Befunde beweisen, welche Vorsicht bei der Anwendung von Analogieschlüssen bei bioch, Prozessen geboten ist, und, dass das Bestreben, aus Überschneidungen von Beobachtungen mit Muskel, Hefe und Bakterien auf die Gleichwertigkeit von biolog. Agenzien zu schliessen, bisher nicht nur experimentell ungenügend untermauert erscheint, sondern Tatsachen an den Tag fördert, die zum Widersprechen zwingen.

Dr. H. A. Krebs (Sheffield) raised the question of the probable identity of Meyerhof's " aldolase " and Neuberg's " carboligase."

JOINT DISCUSSION *with* Section 1 *on* BACTERIAL PHOTOSYNTHESIS

Chairman : Prof. C. H. Werkman (Ames, U.S.A.).

Prof. C. B. van Niel (Pacific Grove, Calif., U.S.A.), **Opener.**

A careful consideration of all the facts and factors which have become known during the last ten years concerning photosynthesis of the green and purple bacteria leads to the conclusion that these processes of photochemical carbon dioxide reduction differ from that which is carried out by the green plants, in that the presence of oxidizable substances is required for the occurrence of the former. Especially the fact that in not a single case has carbon dioxide reduction by bacteria been observed after these oxidizable compounds have been used up lends support to the idea that the recently reported production of oxygen during photosynthesis by purple bacteria (Czurda, 1936) must be explained in accordance with the views developed in 1930.

A certain specificity in the oxidizable substances which various representatives of the groups of green bacteria, Thiorhodaceæ and Athiorhodaceæ, can use in the process of photochemical carbon dioxide reduction has been observed. So far, these observations indicate that the group of green bacteria differs very markedly from the two groups of purple bacteria. The only oxidizable substance which the green bacteria can use is hydrogen sulphide, which is oxidized to sulphur with a corresponding carbon dioxide reduction. The Thiorhodaceæ carry out an oxidation of all the oxidizable sulphur compounds to sulphate, and, in addition, can use molecular hydrogen and various organic substances directly in the photosynthetic reaction. The Athiorhodaceæ are adapted to the use of organic substrates only, although some representatives are undoubtedly also capable of the utilization of molecular hydrogen and hydrogen sulphide.

The differences between Thio- and Athiorhodaceæ seem to be somewhat of the same nature as those between various representatives of each one of the two groups. The ideas expressed recently by Gaffron that photosynthesis of Thiorhodaceæ in the presence of organic substances is the result of two different reactions—the production of hydrogen sulphide from organic sulphur compounds (dark reaction), and the oxidation of hydrogen sulphide in the light reaction—have been shown to be erroneous. The results obtained by Gaffron, which show that Thio- and Athiorhodaceæ behave differently in the presence of organic substances, must be interpreted along other lines. It is mainly the occurrence of a marked acid production by Thiorhodaceæ, even in the absence of substrate, which must be made responsible for the observed differences.

Photosynthesis by all three groups proceeds under the influence of pigments which are responsible for the uptake of radiant energy. Since normal photosynthesis by purple bacteria occurs in the infra-red region (Gaffron), we must conclude that only the green pigments are photochemically involved. It is possible that the red (carotenoid) pigments, occurring in the purple bacteria, fulfil a function as hydrogen transporters, for which the behaviour of some representatives of the Athiorhodaceæ seems to speak.

It is obvious that the different types of photosynthesis now known to occur open up the possibility to attempt to correlate the differences with differences in the organisms. As a first lead, one might inquire in how far the pigments of the photochemical apparatus of the bacteria differ from those of the green plants. The present knowledge of these pigments shows up differences, but as yet these do not seem to account for the different outcome of the photosynthetic process. Quantum efficiency determinations would throw considerable light upon the problem whether also in bacterial photosynthesis, which thermodynamically requires much less energy than green plant photosynthesis, water would be involved. Investigations along this line are being carried out ; the published results do not yet permit, however, of conclusions which seem definite.

*Dr. C. S. French (Berlin) : *The Photochemical Assimilation of CO_2 by Purple Bacteria.*

(*a*) The reduction of CO_2 by butyrate in *Spirillum rubrum.*

The relative absorption curve of the pigment complex was measured photoelectrically by comparing the absorption of suspensions of normal and bleached cells.

The relative absorption curves of the photochemically active pigment, the bacterio-chlorophyll, was determined by the rates of CO_2 uptake (apparent only as a decreased rate of autofermentation) of a thin suspension of the bacteria illuminated with monochromatic light of different wave-lengths and measured intensity. As the reaction in question appeared somewhat difficult to follow quantitatively, attention was turned to another strain which, in contrast to *Spirillum rubrum*, is able to use H_2 instead of a fatty acid for CO_2 reduction.

(*b*) The reduction of CO_2 by H_2 in *Streptococcus varians.*

This reaction occurs rapidly in the light, and only a comparatively small gas production is found in the dark. No utilization of H_2 occurs in the dark. The curve of rate of reaction against H_2 percentage in the gas phase is at half maximum at 4 per cent. H_2. Five per cent. CO_2 is adequate to allow maximal assimilation which is independent of pH between 7·5 and 8·2, though smaller outside these limits. The rate when plotted against light intensity gives a sigmoid curve. Lowering the temperature decreases the apparent inhibiting factor in evidence at low intensities. *Per quanta,*

absorbed by thick suspensions, infra-red light is more effective than visible. Attempts are being made to measure the quantum yield in thin suspensions.

Dr. H. Gaffron (Berlin): *Der Stoffwechsel der Thio- und Athiorhodaceen.*

Schwefelspeichernde und schwefelfreie Purpurbakerien unterscheiden sich auf das deutlichste durch die Intensität ihrer Dunkelreaktionen. Für Photovibrio (Athiorhodacee) ist characteristisch, dass nach Verbrauch der Wasserstoffdonatoren die Kohlensäureassimilation aufhört und danach weder im Hellen noch im Dunkeln ein nenneswerter Gaswechsel auftritt. Für Thiocystis ist charakteristisch, dass zugleich mit der Assimilation von Kohlensäure mit Hilfe zugesetzter Schwefelverbindungen eine Dunkelreaktion einsetzt, in der die ersten Assimilationsprodukte durch eine " Gärung " umgewandelt werden. Bei dieser " Gärung " entstehen Substanzen, die ihrerseits wieder Anlass von Kohlensäure geben, wenn Thiocystis nach einer Dunkelzeit belichtet wird. Van Niel hat nachgewiesen, dass diese Substanz nicht, wie ich angenommen habe, Schwefelwasserstoff ist. Die Bildung von Schwefelwasserstoff im Dunkeln durch Thiocystis muss als eine Nebenreaktion betrachtet werden. Die Bildung der unbekannten Substanz wird durch Kohlenmonoxyd gehemmt. Eine der Dunkelreaktionen der Thiocystis ist deshalb den bekanntlich durch Kohlenoxyd hemmbaren Wasserstoffgärungen verwandt. Dies wird gestützt durch die Tatsache, dass die Purpurbakterien molekularen Wasserstoff für die Kohlensäurereduktion benutzen können.

Die Assimilationswerte (CO_2 assimiliert pro Molekül Substrat) der aliphatischen Fettsäuren bei der Assimilation durch Rhodovibrio steigen in der Weise an, dass sie sich durchschnittlich um 1/3 Molekül Kohlensäure für jede zusätzliche Methylengruppe erhöhen. Merkwürdigerweise findet man den gleichen Unterschied bei Übergang von normaler zu verzweigter Kohlenstoffkette isomerer Fettsäuren. Während die Fettsäure-Assimilation notwendigerweise zu Produkten führen muss, deren Zusammensetzung etwa der aus Rhodovibrio isolierte Polymeren Crotonsäure $(C_4H_6O_2)_n$ entspricht, findet man bei der Wasserstoff-Assimilation das Verhältnis 2 H_2 + 1 CO_2, was auf Kohlenhydrat-Bildung hindeutet. Es erscheint möglich, dass bei der Assimilation der Purpurbakterien mehr als ein *primäres Assimilationsprodukt* gebildet wird.

Dr. F. M. Muller (Amsterdam): *The Metabolism of the Purple Sulphur Bacteria in Organic Media.*

The question whether the metabolism of the purple sulphur bacteria in media containing an organic compound, but devoid of oxidizable sulphur compounds, must be interpreted in terms of a hydrogenation of CO_2 with the organic substrate as a hydrogen donator, may now be considered as settled (*vide* abstract of intro-

duction to the subject of bacterial photosynthesis by Dr. C. B. van Niel).

We shall now deal with another important point in the above-mentioned metabolism, viz., the fate of the organic substrate.

When the p.s.b. are grown under continuous illumination in synthetic media containing one simple carbon compound (e.g., lactic, malic, acetic, succinic or butyric acid), no metabolic products other than CO_2 in quantities varying with the oxidation level of the substrate are found, the substrate being practically completely converted into cell material and CO_2.

It is possible to explain such a metabolism by assuming that the organic substrate undergoes complete dehydrogenation to CO_2 and H_2O, the cell material being built up exclusively from the hydrogenated acceptor CO_2, as in the case of the autotrophic metabolism in organic media containing oxidizable sulphur compounds.

Since, however, dehydrogenation of the substrate probably proceeds by way of pyruvic acid and acetaldehyde, which may be considered as the building stones of the majority of the organic substances constituting the cell material, we may reasonably expect that the dehydrogenation of the substrate generally does not proceed beyond these two compounds.

The formation of CO_2 and organic acids from reserve material in the dark, as found by Gaffron and Roelofsen, constitutes a further important feature in the metabolism of the p.s.b. Probably the same process also occurs besides photosynthesis in the light. As, however, the acids formed in this process in the dark are reassimilated in the light, they will not accumulate in experiments with continuous illumination such as mentioned above.

Dr. E. C. Wassink (Utrecht) : *The Assimilation of Thiorhodaceæ in Monochromatic Light.*

The amount of quanta to be supplied for the assimilation of 1 molecule of CO_2 is at least equal to the energy required for its reduction to carbohydrate level (about three in green plant cells, much less in Thiorhodaceæ, because of the simultaneous dehydrogenation of sulphur compounds). In green plant cells an efficiency of nearly 100 per cent. was found by Warburg. It was of interest to see whether in another group of plants also such a high efficiency would be obtained. On the other hand, at least four quanta would be necessary, if the reduction of a molecule of carbon dioxide proceeds in four subsequent reduction reactions, a possibility discussed in the literature. The advantage of the Thiorhodaceæ over green plant cells in studying this question is the larger margin between the energetically required minimum and 4.

Experiments were made in phosphate buffer mixtures with thiosulphate as a sulphur compound in an atmosphere of nitrogen with 5 per cent. of CO_2. The advantages of phosphate buffers over bicarbonate solutions were shown.

Preliminary determinations of the quantum yield in yellow sodium light and in infra-red sodium light showed the high number of about 40 quanta wanted for the conversion of 1 molecule of CO_2, after correction for the dark metabolism. The energy required for the conversion of carbon dioxide with the aid of thiosulphate is equal to about 2·15 quanta per molecule of CO_2. Besides the possibility that under the conditions chosen not all the pigment present was in an active state, it must not be overlooked (1) that the probably inactive red pigment system may absorb a considerable part of the light (which was not definitely established so far) and (2) that part of the energy taken up may be lost again in collisions of the second kind.

Results of growth experiments in a continuous spectrum were shown, which indicate a strong specificity of growth, beginning in the infra-red and in the yellow, chiefly in accordance with the absorption spectrum of crude green pigment solutions.

Prof. Viktor Czurda (Prague): *Die Stoffwechselvorgänge der Athiorhodaceen.*

Von eigenen Kulturexperimenten legt besonders eine Beobachtung die Entstehung von O_2 im Assimilationsprozess des Chromatium nahe. Ein mit diesem Organismus bisher stets vergesellschaftet angetroffenes Bakterium (Rhodobakterium 23 meiner Athiorhodaceensammlung), das vorzüglich in dem Chromatiumsubstrat gedeiht und auch die von Chromatium vertragene H_2S-Konzentration durch einige Tage ohne Schaden vertragen kann, vermag sich in Reinkultur nur bei einer gewissen unternormalen O_2-Tension zu entwickeln. Es bildet dann rötlichweisse bis karminrote Kolonien. Bei der für die Chromatiumentwicklung notwendigen H_2S-Konzentration unterbleibt seine Vermehrung. Wird es aber mit Chromatium zusammen oder getrennt in einer zweiten angrenzenden Substratschichte eingesät, so erscheinen Kolonien nur dann, wenn der H_2S innerhalb der ersten 8 Tage aufgebraucht ist, was an dem Farbumschlag des Leuko-Indigkarmins zu erkennen ist. Diese Kolonien haben eine bräunlichgrüne Färbung. Eine solche Farbänderung ist auch anderen Athiorhodaceen eigentümlich. Sind in solchen Versuchen viele Kolonien des Rhb. 23 vorhanden, dann bleibt diese Färbung wochenlang erhalten. Sind hingegen wenige Kolonien entwickelt, dann geht nach einiger Zeit die anfänglich bräunlichgrüne Färbung in die rote über, welche sonst nur bei O_2-Gegenwart auftritt. Die durch den Indikator angezeigte Redoxpotential-änderung scheint demnach doch auf der O_2-Bildung zu beruhen, wie in den Versuchen mit grünen Plfanzen. Die Stoffwechselvorgänge möchte ich daher in folgendes Schema fassen:

1. $4CO_2+4H_2O=4CH_2O+4O_2$
2. $2H_2S+O_2=2H_2O+2S$
3. $2S+3O_2+2H_2O=2H_2SO_4$

Damit ist den Beobachtungen des gesamten Stoffumsatzes (van Niel) und der Feststellung nicht messbarer CO_2-Aufnahme bei der blossen S-Verarbeitung (Roelofsen) und dem Verhalten des Rhb. 23 so wie einigen hier übergangenen Erscheinungen Rechnung getragen. Auch die "Autoassimilation" wäre verständlich gemacht durch die Möglichkeit der Annahme der Verarbeitung von CO_2 und H_2S in zwei getrennten unabhängigen Prozessen. Schliesslich würde die "Autofermentation" ohne Schwierigkeiten als eine schwache O_2-Atmung der fakultativ anaeroben Chromatien aufgefasst werden können.

Dr. F. M. Muller (Amsterdam).

Die Resultate von Herrn Czurda mit Stamm 23 in Reagensgläsern mit Mineralsalzagar können vielleicht in folgender Weise gedeutet werden.

Die Tatsache, dass die Bakterien sich in dem Glase, das eine Schicht Agar enthält, nur zwischen 5 und 15 mm unterhalb der Oberfläche entwickeln, deutet darauf hin, dass die Bakterien empfindlich gegen höhere Konzentrationen von H_2S sind. Von der Oberfläche her diffundiert Sauerstoff in den Agar und oxydiert den H_2S, wodurch die Konzentration herabgesetzt wird.

In dem Glase mit drei Schichten Agar, von denen die untere Keime vom Stamm 23, die mittlere Keime von Chromatium und reduziertes Indigkarmin und die obere keine Keime enthält, fangen die Keime vom Stamm 23 erst an sich zu entwickeln, wenn sich das Chromatium schon voll entwickelt hat, und die Farbe des Indigkarmins zu erscheinen anfängt. Durch die Entwicklung des Chromatium wird der H_2S in der mittleren Schicht erschöpft und H_2S wird von unten nachdiffundieren, was ebenso eine Herabsetzung der H_2S-Konzentration im Milieu des Stammes 23 bedeutet.

Die Oxydation des Leuko-Indigkarmins wird nicht durch Sauerstoff, der von oben her in die mittlere Schicht hineindiffundiert (die obere Agarschicht ist ja an ihrer Oberseite mit Paraffin verschlossen) sondern von dem Chromatium in der mittleren Schicht verursacht. Wie Herr van Niel berichtete, sind die roten Schwefelbakterien imstande, den Wasserstoff aus Leuko-Indigkarmin, ebenso wie den Wasserstoff aus H_2S auf CO_2 zu übertragen. Eine Blaufärbung der mittleren Schicht besagt alsdann nur, dass alles H_2S aus dieser Schicht zu H_2SO_4 oxydiert worden ist und die Chromatien angefangen haben, den Leuko-Farbstoff als H-Donator für ihre Photosynthese zu benutzen.

Prof. V. Czurda (Prague).

Zur Veranschaulichung der bisher unter den Athiorhodaceen angetroffenen Mannigfaltigkeit wird das morphologische und kulturelle Verhalten der auffallendsten Stämme (*Rhodobacterium* 1,

9, 16, 14, 23, *Rhodospirillum* 1, *Rhodospirillum rubrum* und Chlorobacterium 1) in Pepton- und Mineralsalzlösungen, unter aeroben und anaeroben Bedingungen, im Licht und im Dunkeln mit und ohne CO_2 und H_2S geschildert. Das in den Abstracts (S. 267) geschilderte Verhalten des Rhb. 23, vor allem die Entwicklung in drei-Schichtkulturen mit Chromatium nach Umschlag des Indikators, wurde dort ähnlich wie früher der Indikatorenumschlag auf O_2-Entstehung im Assimilationsprozess des Chromatium zurückgeführt. Diese Vorstellung gebe ich auf, weil die hier geschilderten Kulturbeobachtungen einer Erklärung ohne O_2-Entstehung im Sinne der van Niel'schen Vorstellung restlos zugänglich sind.

DISCUSSION *on* INFLUENCE OF SUBSTRATE ON THE CHEMICAL POTENTIALITIES OF THE CELL*

Chairman : Sir Arthur Harden (London).

Dr. J. H. Quastel (Cardiff), **Opener** : *Bacterial Enzyme Formation as a Function of the Nutritional Medium.*

Experiments have been carried out to determine whether enzyme production bears any definite relationship to the nature of the medium on which the organism is grown.

The organism studied has been *M. lysodeikticus*. This was chosen because suspensions of the organism are easily lyzed by dilute egg white. Such endocellular enzymes as catalase, urease and fumarase can be estimated with accuracy in the lyzed suspensions. With non-lyzed suspensions, on the other hand, it is difficult to make relative estimates of the content of enzymes owing to the possibilities of interfering factors, e.g., changes of cell permeability. A study of the relative contents of catalase, fumarase and urease in *M. lysodeikticus* shows that these enzymes vary very greatly with respect to each other according to the medium on which the organism is grown. The presence of glucose in an agar-peptone medium appears greatly to stimulate the formation of urease and to depress that of catalase. The presence of urea in an agar-peptone medium does not stimulate urease formation, nor does that of succinate or fumarate stimulate fumarase production. Fumarase production is relatively high when the organism is grown on a glucose-agar-peptone medium. The capacity of the cell to oxidize lactic acid also varies with the nature of the medium.

These results are best interpreted by assuming that the enzymes are themselves metabolites normally undergoing changes in the cell, whose rates of formation and destruction vary with the conditions of growth.

* In the absence of Prof. H. von Euler (Stockholm), the Discussion was opened by Dr. J. H. Quastel.

Whether a substrate brings about enzyme enrichment, or impoverishment depends on (*a*) whether it, or its breakdown products, tend to destroy the enzyme, or to preserve it (e.g., by a reversible combination with the enzyme) from further breakdown; and (*b*) whether it influences, in a general way, the synthesis in the cell of certain classes of enzyme by contributing the necessary organic molecule for synthesis.

Prof. Cl. Fromageot and **Dr. Paulette Chaix** (Lyon): *Action des dérivés sulfurés sur la fermentation bactérienne du glucose.*

Des travaux précédents[1] ont montré que des bactéries propioniques (Propionibacterium II) sont rigoureusement incapables, dans des conditions déterminées, d'attaquer le glucose si elles se trouvent en quantités inférieures à la *quantité active limite*, mais que l'addition de certains dérivés sulfurés leur rend l'activité qu'on leur fait ainsi perdre par simple dilution. Une étude systématique, à ce point de vue, de différentes substances susceptibles de fonctionner en activateurs, ($t° = 37°$; $pH = 6.4$ atmosphère de CO_2; mesures manométriques) a donné les résultats suivants:

1° Activité maxima (correspondant à $Q^{CO_2}_{CO_2} = 20$ à 25):

H_2S, acides thioglycolique et thiolactique, thiourée, thioacétamide, cystine, cystéine, méthionine, glutathion (réduit et oxydé), disulfure de tétraméthylènethiurane.

2° Activité nulle ($Q^{CO_2}_{CO_2} = 0{,}00$):

Soufre précipité lavé, sulfate de potassium, sulfocyanure de potassium, taurine, bleu de méthylène, insuline, alanine, glycocolle, acétate d'ammonium, les colorants dont la liste est plus loin.

3° Activité partielle ($Q^{CO_2}_{CO_2} = 7$ à 14):

Hyposulfite de sodium (14), thioindoxyl (9).

Les colorants suivants: phénol-indo-2-6-dichlorophénol, bleu de toluène, bleu de méthylène, indigotrisulfonate de K, indigodisulfonate de K, bleu Nil, vert Janus, phénosafranine, rouge neutre, n'ont aucune action sur la vitesse de la glycolyse, que cette vitesse soit nulle, ou qu'elle ait sa valeur maxima en présence de l'un des activateurs.

D'autre part, l'action des bactéries en petites masses sur ces mêmes colorants montre que la déshydrogénation du glucose, contrairement à la glycolyse, ne nécessite pas la présence des activateurs en question.

***Prof. C. Gorini** (Milan): *L'influence du substrate sur les potentialités chimiques de la cellule.*

(1) *Influence du substrate sur les potentialités protéolytiques des bactéries*:—On discute si les bactéries produisent les enzymes en

[1] C.R. Ac. Sc. **201**, 626, 857; 1935 et **202**, 963; 1936.

dépendance de leur besoin, de sorte que la qualité des enzymes serait variable suivant le substrate. Mes recherches sur les potentialités caséolytiques des bactéries répondent négativement. En effet dès 1892-94 (Riv. Igiene Sanità, 3, 527, 1892 ; 4, 549, 1893—Giorn. R. Soc. It. Igiene, 16, 129, 1894) j'ai démontré que le *B. prodigiosum* et d'autres bactéries produisent la chymase non seulement dans le lait mais aussi en absence de lait et de caséine (gélatine, gélose, etc.). En 1926 (C.R.Ac.Sc. 1926, 182, 946 ; 183, 250, et 681 ; 184, 1355) j'ai démontré que les potentialités caséolytiques ne sont pas suscitées mais simplement stimulées par des catalysateurs (peptone, sang, vitamines). Enfin en 1927-32 (Journ. Path. Bacter., 30, 505, 1927, et 35, 637, 1932) par une méthode spéciale de culture ("lait-suragarculture")[1] je suis arrivé à démontrer que les potentialités caséolytiques sont très répandues chez les microbes, bactéries, levures et actinomycètes, même chez des espèces qui sont considerées inactives sur le lait (*B. typhi*, *Str. equi* et *equinus*, *Strept. epidemicus* Minett, *Str. infrequens* et low acid *Str.* Stableforth, *Str. scarlatinæ* Allison, *Str. tonsillitidis* Griffith, *Str. mucosus lyticus* Dudgeon, *Sacchar. ellipsoideus* Hansen, *Actin. aureus* Waksman, etc.).

En conclusion : le substrate exerce certainement une influence sur les potentialités caséolytiques bactériennes, mais il s'agit plutôt d'un effet quantitatif que qualitatif.

(2) *Les substrates sucrés épargnent les proteines* :—Aussi l'action protectrice des substrates sucrés sur les protéines semblerait une épreuve que les enzymes bactériens sont produits en réponse du besoin. En effet certaines bactéries, comme je l'ai démontré pour le vibrion cholérique dès 1893 (Giorn. R. Soc. It. Igiene 3, 796, 1893), en présence de sucres, utilisent ceux-ci de préference aux protéides :—Mais cela ne signifie point que dans ce cas ils ne forment pas de protéases ; c'est que, par la fermentation des sucres, le *p*H du substrate devient défavorable au fonctionnement des protéases de ces bactéries, qui sont alcalino-protéolytiques ; c'est l'explication qui derive des doctrines modernes enzymologiques.

***Dr. H. Karström** (Helsinki) : *The Problem of Adaptation in Bacteriology.*

It is known that a given species of a micro-organism acts upon quite definite chemical compounds, owing to the fact that the organism in question is capable of producing quite specific enzymes which are characteristic of that species. According to the conditions for their formation, the specific enzymes of a given micro-organism can be divided as follows :—

(1) *The constitutive enzymes*, which are always developed by a given micro-organism irrespective of the composition of the medium on which the organism grows.

[1] Cette méthode consiste en ceci, que, au lieu d'ensemencer les microbes directement dans le lait additionné de stimulants on ajoute du lait aux cultures déjà bien dévéloppées sur gélose additionnée de stimulants.

(*a*) The constitutive enzymes, which are always developed in equal quantities, irrespective of the medium.
(*b*) The constitutive enzymes, which are developed in greater quantities when the micro-organism grows on the specific substrate.
(2) *The adaptive enzymes*, which are developed by a micro-organism only when the organism grows upon the specific substrate.

The author has never found that a micro-organism could be induced, through what Yudkin calls "training," to form enzymes which are foreign for the species. According to the author's experience the formation of an adaptive enzyme by a species of micro-organism is invariably associated with the life phenomena of the cells. A non-living cell-mass never produces enzymes that were not contained in the living cells before the killing. If a cell-mass, in which the multiplication of the cells has been rendered impossible by any specific treatment, is nevertheless found to produce adaptive enzymes the explanation must be that such cells are not completely devoid of all life activities although they have lost their ability of cell-division. The adaptive enzyme formation should be duly considered when enzyme preparations are made from micro-organisms, e.g., for specificity studies. Such a biological enrichment and "purification" of enzymes is in many respects valuable in enzymology.

Working with various strains of *Bact. coli* and the lactic acid bacteria, the author has shown that, whenever the cells of these organisms ferment maltose or lactose, they also produce the corresponding enzymes, maltase and lactase. *Direct* fermentation of these disaccharides, without a preliminary enzymic hydrolysis—as assumed by *Willstätter* for certain yeasts—does not take place in bacterial processes.

Dr. W. R. Wooldridge (London): *The Influence of Substrate on the Chemical Potentialities of the Cell.*

Various authors have reported increased activity of certain enzymic systems in yeasts and bacteria when these organisms have been grown on the substrate of the system examined. Such results, although probably true, must be carefully scrutinized, for it has been shown, working with bacteria, that the activities of the dehydrogenases may vary considerably with a number of factors. The time the organism is allowed to grow or remain in the nutrient medium before it is reaped for examination very considerably affects the activity of certain enzymes whilst altering others to a much less degree, e.g., the organism examined after 12 hours' growth may exhibit an enzymic picture totally different from the picture shown by the 72-hour organism. This variability is not directly dependent upon the number of viable cells present in the suspension, although

viability may be one of the factors having some influence on enzymic activity. The presence of co-enzymes and the actual condition of the organism, e.g., whether it is reaped in the logarithmic phase of growth or not, seem to be more important. The substrate of the culture medium influences the enzymic activity and usually results in the corresponding enzyme retaining its activity beyond the time when most other dehydrogenases have considerably lost their activities, so that compared with these other enzymes it appears to have increased in activity. This result is frequently an artefact due to the more rapid loss in activity of the other enzymes rather than to an increased activity of the "homologous" enzyme. Nevertheless, it would appear that the activity of, or the concentration of, a "homologous" enzyme is frequently increased when the organism is grown upon the substrate for that enzyme.

It is suggested that these and other results of changing activities in cells can best be explained by picturing the cellular surfaces as comparatively loosely-knit structures composed largely of complex organic molecules, the latter orienting themselves in the surface as the result of the affinities exhibited between the various groupings of that molecule and those possessed by the adjacent molecules in the surface and the molecules in true solution on either side of the surface. As the final activities exhibited by the surface will depend largely upon the nature and arrangement of the groupings available at the surface, anything which tends to rearrange or mask this disposition of groupings may alter some vital activity. It is suggested that this arrangement is particularly prone to be altered somewhat during the phases of cellular growth and division, and that if a particular substance is present in high concentrations in the fluid bounding a surface, some surface molecules may be re-oriented so that more groupings active for that substance become available. In this way a substrate present during the growth of a cell will tend to increase the concentration of enzymes available to act on that substrate.

Mr. L. Bradley Pett (Toronto): *Observations on Yeast grown in Cyanide.*

In spite of the long-known poisoning effect of cyanide, the observation has been made that yeast can be grown in cyanide concentrations up to *M*/500. Frequent additions of cyanide to the acid medium are necessary, and a method of continuously bubbling with HCN-laden air has been devised. Controls treated by air and by nitrogen have been used, and the properties of the resulting yeasts are being studied.

The flavin content of cyanide-yeast is 2-3 times higher than that of aerobic or anaerobic yeast. Observations have been made on the respiration of the three yeasts in various substrates. Cyanide-yeast has a very low respiration in glucose and other substrates, and this is

not greatly inhibited by cyanide. The aerobic fermentation of cyanide-yeast is higher than the fermentation of aerobic yeast, measured either aerobically or anaerobically, but is less than that of anaerobic yeast. The Pasteur effect is not demonstrable. The effect of continuous subculturing in cyanide indicates the possibility of developing a strain which will remain constant with or without cyanide.

Cyanide-yeast seems to be a new yeast type of low respiration, high aerobic fermentation, and occupying a position in some respects between aerobic (baker's) and anaerobic (brewer's) yeast, but distinct from either.

Dr. J. Yudkin (Cambridge): *Enzyme Variation in Micro-organisms.*

The enzymic constitution of micro-organisms is often dependent on the conditions under which they are grown. The presence of certain substances in the medium frequently evokes the production of enzymes, not otherwise present, which are able to act upon these substances. Such enzymes are called by Karström "adaptive," those which are always present being called "constitutive."

There are two possible methods by which a substrate could produce in a growing culture of micro-organisms its specific enzyme. First, there may always be present in a culture a small number of organisms possessing the enzyme, arising perhaps as a mutation. The presence of the enzyme substrate in the medium would presumably place such organisms at an advantage, and these would be selected out. The resulting culture would then contain a majority of such organisms. Secondly, the substrate may have a direct effect on the enzymic make-up of the individual cells. This would be as a direct response to an alteration in the external environment, and may be compared with the production of acquired characters in higher organisms. Production of these two types of enzyme variation have provisionally been termed "training" and "adaptation" respectively.

The *mutabile* strains of *Bact. coli*, in which cultures unable to ferment lactose gradually produce lactose-fermenting colonies on this sugar, may be taken as examples of natural selection or training. Lewis recently showed that in such a strain of *Bact. coli mutabile*, lactose-fermenting mutants are continuously being produced, even in the absence of lactose, and that the effect of a lactose medium is thus only to select out such variants.

On the other hand, in two cases of enzyme variation, it has been possible to show quite clearly that the production of the new enzyme occurs as a result of the interaction of the cell and the substrate. These enzymes are the hydrogenlyase of *Bact. coli* and the galactozymase of yeast. In both cases, enzyme formation was obtained in the absence of cell division, that is, in conditions in which natural selection was impossible.

Dr. Marjory Stephenson and **Dr. Caecilia Lutwak-Mann** (Cambridge): *The Effect of Substrate on some Enzymes of* Bact. coli *etc. connected with Fermentation.*

Glycolysis and hydrogen production by washed suspensions of cells of *Bact. coli* are influenced unequally by 1, aerobic conditions, and 2, the nature of the substrate during previous growth. Hence glycolysis cannot be measured by gas production.

Glycolysis was measured in $Q_{hexose,}$ i.e., γ-hexose mg/ dry wt. organism/hour; hydrogen production in Q_{H_2}. Four degrees of aerobiosis were used; O. Complete anaerobiosis; 1. Flask closed by plug; 2. Liquid medium in Roux bottle; 3. Surface of agar in Roux bottle. 0, 1 and 2 do not significantly affect $Q_{glucose}$ but give decreasing values of Q_{H_2} (78, 52 and 15 respectively), whilst $Q_{glucose}$ remains between 450 and 600; 3 causes a drop in $Q_{glucose}$ to 245 and a further drop in Q_{H_2} to 8. With conditions of aeration kept constant at 3, growth in absence of glucose (0.5 per cent.) gave $Q_{glucose}$ 245, growth in presence of glucose gave $Q_{glucose}$ 445. At aeration 1, growth without glucose gave $Q_{glucose}$ 505, growth with glucose $Q_{glucose}$ 1125.

As in the case of certain yeasts galactozymase in *Bact coli* is adaptive. Growth on plain broth (aeration 1) ferments both sugars, $Q_{glucose}$ 500 $Q_{galactose}$ 240; the presence of either glucose or galactose in the broth raises the $Q_{glucose}$ about equally, 1130 and 1,000 respectively, but glucose depresses the $Q_{galactose}$ to 25 whilst galactose raises $Q_{galactose}$ to 425.

A similar effect of medium is observed on the oxidation of the two sugars; thus the Q_{O_2} (glucose) was 218 when grown on glucose broth and 210 when grown on galactose broth, whilst growth on glucose broth gave a Q_{O_2} (galactose) of 46 and growth on galactose broth a Q_{O_2} (galactose) of 213.

The effect of glucose in lowering and of galactose in raising the fermenting power of *Bact. coli* towards galactose is comparable to the effect of glucose on washed suspensions of *S. cerevisiæ* adapted to ferment galactose. (Stephenson and Yudkin, *Biochem. J.* 30, 56, 1936). Here the cells lose their power of fermenting galactose when removed from this sugar and allowed to ferment glucose, but regain it on washing free from glucose and leaving in contact with galactose. In both cases adaptation to glucose appears to decrease or remove power to ferment galactose.

Dr. E. R. Dawson (Epsom, Surrey): ***Enzyme Formation.***

Dr. Quastel's suggestion that a substrate brings about enzyme enrichment or impoverishment according as it or its breakdown products tend to preserve or destroy the enzyme is of interest in connection with the formation of invertase and maltase by yeast. Under certain conditions the invertase content of yeast can be increased several fold by propagation in a medium containing sucrose or invert sugar. Similarly maltose increases the maltase content. Growth in glucose, however, decreases the maltase content. The different response of the two enzymes to the presence of breakdown products may be connected with affinities. Invertase has a greater affinity for sucrose than for glucose or fructose; maltase on the other hand has a much greater affinity for glucose than for maltose.

When considering the different response of adaptive and constitutive enzymes to specific substances in the growth medium, it should not be forgotten that the environment of the enzymes themselves is not the medium in which the cell is growing, but the cell contents as already modified by other catalytic systems.

SECTION 8

SPECIFIC IMMUNIZATION IN THE CONTROL OF HUMAN AND ANIMAL DISEASE

DISCUSSION *on* THE CONTROL OF DIPHTHERIA AND WHOOPING-COUGH BY MEANS OF SPECIFIC IMMUNIZING REAGENTS

Chairman : Prof. W. H. Park (New York).

A.—DIPHTHERIA.

Prof. W. H. Park (New York), **Opener** : *The Control of Diphtheria by Specific Immunization.*

The control of diphtheria by specific treatment and specific prophylactic immunization is one of the most gratifying victories of bacteriology. In 1894, the death rate from diphtheria was 150 per 100,000 in the City of New York ; it had gradually dropped by 1914 to 20 per 100,000, and by 1935, due to extensive prophylactic immunization, to 1 per 100,000.

In experiments in which one group of guinea-pigs was inoculated with alum-precipitated toxoid (A.P.T.), and another with formol-toxoid (F.T.), the former produced on the average 5 to 15 times more antitoxin than the latter. A single dose of A.P.T. was superior to two or three doses of unmodified toxoid at 1, 2 and 3 months after immunization. Two or three doses of any toxoid gave a higher percentage of Schick negatives than a single large dose of the same preparation.

Previously immunized children who had lost their immunity were more responsive to additional immunization and retained it longer than those who were not immunized before.

In human subjects, the duration of immunity produced by one dose of alum precipitated toxoid as compared with two doses of unmodified toxoid varied considerably with different investigators ; tables of the results obtained are discussed. In our own work in New York City, of the children immunized with one dose of A.P.T., or three doses of T.A.M., 95 per cent. were Schick negative 2 to 3 years later ; of those immunized with two doses of F.T. only 86 per cent. were still negative after this period. In this investigation the average antitoxin content of the blood after A.P.T. was 0.115 units, after T.A.M. 0.036 units and after F.T. 0.026 units.

Undoubtedly, further research is needed in order to determine the duration of immunity obtained by the various methods which are in use at present for diphtheria immunization. Two or three doses of A.P.T. should be tried for achieving lasting immunity in young children in whom severe reactions to the injection of 0.2 and 0.4 cc. doses are not common. In tabulating the results the number of flocculation units should be given in each experiment so that the work of various investigators may be compared. For a comparative study only Schick positive cases which did not have previous

immunization should be used. The determination of the antitoxin content of the blood both before and at various intervals after immunization would make the experiment more accurate than Schick testing alone.

Dr. G. Ramon (Garches). [Communicated by Prof. M. Weinberg, (Paris.)]: *L'anatoxine diphtérique et les vaccinations associées dans la prophylaxie de la diphtérie.*

Depuis longtemps étudiée expérimentalement par divers chercheurs puis effectivement tentée chez l'homme lui même, par Behring et William Park au moyen des mélanges de toxine et d'antitoxine, la vaccination antidiphtérique n'a véritablement pris son essor qu'à partir de la mise en évidence de l'anatoxine diphtérique (1923).

Aus mélanges "toxine-antitoxine," d'une innocuité relative, imprécis dans leur composition, irréguliers et inconstants dans leur action, allait se substituer rapidement l'anatoxine, dont l'innocuité est par définition et en réalité absolue et dont les diverses propriétés sont définies, fixes, stables. Grâce à la réaction de floculation (G. Ramon, 1922) qui après avoir largement contribué à sa mise en évidence allait servir à sa mesure, l'anatoxine peut être évaluée dans son pouvoir antigène intrinsèque, indice de son activité immunisante.

Des recherches poursuivies au laboratoire ont permis d'accroître peu à peu la valeur antigène intrinsèque de l'anatoxine (G. Ramon, Glenny, Schmidt) et de ramener de trois à deux le nombre des injections vaccinales (G. Ramon et R. Debré). La vaccination par injection unique n'immunise qu'un pourcentage insuffisant d'individus.

C'est en 1923 que fût faite, chez l'homme, la première injection vaccinale au moyen de l'anatoxine diphtérique dont l'innocuité pour le sujet humain était ainsi prouvée (G. Ramon).

La méthode s'est bientôt répandue et généralisée. A l'heure actuelle elle est universellement répandue.

En fait, les résultats déjà acquis dans la plupart des pays ont consacré l'efficacité de la vaccination au moyen de l'anatoxine diphtérique.

Ils ont affirmé l'exactitude des principes qui ont présidé à l'élaboration à la mise au point de cette méthode d'immunisation et qui, dans la suite ont servi de guide aux progrès effectués.

Ils entrainent la conviction de voir disparaître la diphtérie par la pratique systématique et précise de cette méthode de prophylaxie qui déjà, à l'heure actuelle, partout où elle a été correctement mise en œuvre a permis une réduction le plus souvent considérable de la morbidité et de la mortalité diphtériques.

La vaccination par injection unique d'anatoxine concentrée ou additionnée d'alun ne doit pas être d'un usage courant, du moins dans

l'état actuel de nos connaissances. Elle ne confère l'immunité qu'à un pourcentage en général trop faible de sujets vaccinés. Elle donne une fausse sécurité.

La vaccination antidiphtérique peut-être réalisée selon la formule des " vaccinations associées " (par mélange de l'anatoxine diphtérique avec des vaccins microbiens ou avec d'autres anatoxines, et cela dans les meilleures conditions de commodité et d'économie, avec le maximum d'efficacité.

Prof. B. Johan (Budapest) : *Immunization against Diphtheria in Hungary.*

An organized campaign against diphtheria began in 1929 with the search for and isolation of diphtheria carriers. This had no noticeable influence on the incidence of the disease.

Formol toxoid was then used for immunization on a large scale ; 90 to 98 per cent. of children became Schick negative. The morbidity rate was reduced to one-tenth of that of non-immunized children. Three injections were necessary to obtain a high grade of immunity, but it was difficult in rural regions to get the children to attend for the three injections.

Later a " one-shot " method with alum toxoid was substituted, 20 Lf units being injected. In animal experiments it was found that the " depôt " kept its antigenic property for 32 to 38 days. The reactions did not differ from those caused by the formol toxoid ; cold abscesses were observed only at the rate of 3 per 10,000 injections. 93.3 per cent. of 2,556 Schick positive children, aged 2 to 12 years, were Schick negative two months after a single dose of 1.0 cc. One year and two years after the inoculations the Schick reactions were still negative in over 90 per cent. 90 per cent. of 102 children tested for antitoxin content two years after immunization had more than 0.01 antitoxin units per cc.* Children with such a level of circulating antitoxin all showed a negative Schick reaction.

The immunity was not lower after " one shot " of alum toxoid than after three injections of formol toxoid.

800,000 children in Hungary have been fully immunized with formol toxoid or alum toxoid ; the total number of children under 12 years of age is 1,800,000. Charts were presented showing that the diphtheria morbidity for (*a*) districts in Hungary itself and (*b*) Hungary and neighbouring countries was reduced in inverse proportion to the amount of immunization. The age distribution of diphtheria cases also demonstrated the good result of immunization.

On the basis of laboratory and experimental studies performed at the State Hygienic Institute of Hungary, especially by Professor Tomcsik and Dr. Faragó, the Hungarian Parliament has passed a law which enables diphtheria immunization to be made compulsory. From January 1st, 1937, the Minister of the Interior will bring the law into force for certain age groups.

Prof. J. G. FitzGerald, Prof. D. T. Fraser and **Dr. N. E. McKinnon** (Toronto). [Communicated by Dr. N. E. McKinnon.]: *Attempts to Control Diphtheria in Canada.*

Diphtheria has constituted one of the most important public health problems in Canada. The extensive use of toxoid in three doses has reduced both the morbidity and the mortality from diphtheria; the practicability of its use in controlling diphtheria is now established. The reaction test has proved a valuable aid in detecting and thus avoiding possible reactions. The Schick test used in different countries is by no means standardized and requires further investigation.

The observations in Canada show that immunization of part of a community reduces the hazard in the unvaccinated. No support has been found for the suggestion of Dudley that the specific prevention of diphtheria may be followed by an increase in the diphtheria carrier rate in any community.

Two or three doses of unmodified toxoid give a significantly higher and more durable immunity than one dose of alum precipitated toxoid. While not maintaining that the ideal procedure for diphtheria prevention has been developed, it is evident that substantial progress has been made.

Dr. R. A. O'Brien (Beckenham, England): *The Control of Diphtheria.*

The following prophylactics have been employed:—

(1) *Toxin-antitoxin mixture*—Park, New York.

(2) *Toxoid-antitoxin mixture*—used in England from early days, and still much used. Small liability to cause reactions; reasonably high immunizing power.

(3) *Formol toxoid.* Introduced for human immunization by Ramon (1923); moderate potency. Toxoid of 60-90 Lf units made available by Pope; undesirable reactions have restricted use in England; Moloney test unpopular.

(4) *Toxoid-antitoxin floccules.* "Purest" toxoid available; little reaction; high immunity; used for older subjects in England.

(5) *Alum precipitated toxoid* (Glenny, Pope and colleagues). Troublesome reactions in some adults or adolescents. Used mostly for children. Single injection immunizes where some basal immunity. In guinea-pigs, two doses, e.g., 0.1 cc. followed by 0.5 cc. or less, three weeks later, immunize better than 1.0 c.c (Glenny). In human subjects, similar results obtained (Chesney, Parish). Tendency in England is to inject 0.1 cc. to "detect" (and eliminate for further immunization with T.A.F.) children likely to suffer reactions from larger doses. If no reaction to 0.1 cc., second small dose of A.P.T. given three weeks later.

Immunization has protected, even against "gravis" infection, nurses tending severe diphtheria, and subjects artificially immun-

ized so that circulating antitoxin high. Further experience may indicate necessity for more intense course of injections for general population. But in Dudley's classical epidemic, ordinary immunization provided high protection in school heavily invaded by "gravis" strains.

Dr. G. Chesney (Poole, England) : *A Simple and Efficient Method of Diphtheria Immunization.*

In diphtheria immunization, any procedure which can be recommended for general use on a wide scale should be simple, safe and efficient. The necessity for skin tests should eventually be eliminated.

During the past fifteen months I have used two small doses of Alum Precipitated Toxoid, given subcutaneously, at an interval of four weeks, and have obtained a Schick negative rate of virtually 100 per cent. after immunization. Reaction difficulties have been minimal and negligible.

Over 500 children, aged 1 to 14 years, have been treated, the initial dose being 0.2 cc. (0.1 cc. in children over 10 years), and the final dose 0.4 cc. The small initial dose has served as a "detector" of hypersensitive persons, and the second dose has been reduced when indicated by the occurrence of any local reaction after the first dose. In only one case has it been advisable to complete immunization with T.A.F.

Three hundred of these children have been Schick-tested within two months of the second dose, and in only one case was a positive reaction obtained. This case, on re-test a month later, was found to be negative. A fourfold toxin, indicating a higher level of immunity than that of the standard Schick toxin, has been used in Schick-testing 117 of these cases after immunization. The Schick relapse rate has not yet been determined.

The two dose method has eliminated the necessity for Moloney-testing and Schick-testing after immunization, and is a simple and highly efficient procedure.

Surgeon Rear-Admiral S. F. Dudley (London) : *Principles in the Control of Diphtheria.*

Latent immunization, i.e., the increase in antitoxic immunity which is acquired naturally by carrier or unrecognized infection with *C. diphtheriæ*, has an important effect on the artificial induction of antitoxic immunity. For instance, the lower the percentage of Schick immunes there are in a natural population group, the harder will it be to immunize the Schick susceptible fraction of that group by anti-diphtheria inoculations; because, on the average, the amount of latent immunization a group has undergone is proportional to the percentage of Schick negative reactors it contains. The

natural process of active immunization determines to some extent the duration of Schick immunity. Many natural or "artificial" Schick immunes appear to require frequent contact with toxigenic *C. diphtheriæ* in order to maintain the amount of circulating diphtheria antitoxin in their blood above Schick level.

Artificially immunized subjects are as liable to carrier infection as are natural Schick immunes. Therefore, under certain circumstances, *half-hearted anti-diphtheria campaigns may exert no* favourable effect on the total incidence of diphtheria, and may even increase the risk of infection to the unprotected residue of children in the community.

Although some "gravis" types of *C. diphtheriæ* can break through antitoxic immunity and occasionally cause a severe illness in Schick immune subjects, yet in general the modern diphtheria *prophylactics will protect against "gravis" diphtheria, or, at the* most, only allow a trivial transient illness to develop.

Dr. G. Bousfield (London): *The Control of Diphtheria.*

Most types of prophylactic were efficient if the optimum dosage and intervals were determined. Reactions were more common after A.P.T. and F.T. than after T.A.M. or T.A.F. Goat T.A.M. produced more reactions than horse T.A.M. There was a gain of 9 per cent. immunizing efficiency when a third dose of 1.5 c.cm. of Burroughs Wellcome T.A.M. was given instead of 1 c.cm. A.P.T. (Burroughs Wellcome) has proved a highly efficient antigen in an urban population, given as two injections of 0.1 and 0.5 c.cm. (14 days interval). One injection has yielded poor results in rural areas. In children, T.A.F. has no advantages over T.A.M., either from the antigenic or "freedom-from-reaction" aspect. For adults, T.A.F. has proved most suitable. More thorough immunization should be attempted, to protect against the most virulent types of K.L.B. A dosage of 0.1, 0.3 and 0.5 c.cm. of A.P.T., at intervals of 14 days was suggested.

Cases of allergic reactions to the Schick test were described. One was mild, causing only œdema of the face, eyelids, lips and neck. The other case showed marked œdema in the same situations ten minutes after a post Schick test. Considerable respiratory distress, cyanosis and rapid pulse were features. Recovery in 1½ hours followed the injection of adrenaline. Skin tests showed that this child had developed an allergy to Witte's peptone, the stabilizer in the test toxin. It might be desirable to discover a different toxin stabilizer.

Manufacturers should inform doctors in concise language of what can fairly be expected of diphtheria prophylaxis. If lay persons were misled, there might be delay in seeking medical aid if diphtheria attacked an imperfectly immunized person, possibly with fatal results.

Prof. R. Prigge (Frankfurt a. M.).

Die Unterschiede zwischen den mit Alauntoxoiden erzielten Resultaten erklären sich durch die Tatsache, dass diese Präparate ohne vorherige Messung ihres Immunisierungswertes verwandt werden. In Deutschland müssen daher die Alaun-toxoide ebenso wie die übrigen Diphtherieimpfstoffe staatlich geprüft werden.

Die deutsche Prüfungsmethode beruht in 1. Linie auf der Erkenntnis, dass die Wirkungskurven der biologisch aktiven Präparate *Aufzählungskurven* sind und durch eine einfache mathematische Operation in *Verteilungskurven* umgewandelt werden können (Kisskalt 1915) ; 2. auf der Einsicht dass der *einfache Reihenversuch* fehlerhafte Resultate liefert, wenn infolge der *individuellen Unterschiede zwischen den Versuchstieren* die Differenz zwischen der absolut wirksamen und der absolut unwirksamen Dosis einen bestimmten Wert überschreitet (Prigge u. Hartoch, 1930). 3. auf der wohlfundierten Theorie, dass die *Aufzählungs-* und *Verteilungs-kurven* " normal " werden, wenn man anstelle der Dosen deren Logarithmen als Abszissen einführt (Gray, Trevan, Bainbridge and Attwood, 1931). Mit Hilfe dieser Methodik konnte nachgewiesen werden, dass die *Immunisierungskraft* der einzelnen Alauntoxoide *ausserordentlich verschieden* ist. Der Grund hierfür ist darin zu erblicken, dass der Immunisierungswert (gemessen in Schutz-Einheiten, SE) zwar mit der Quadratwurzel der Flockungs-Einheiten (Lf) steigt, aber ausserdem von einem variablen unspezifischen Koëffizienten (K) abhängt :

$$\text{SE ccm.} = \text{K} \sqrt{\text{Lf ccm.}}$$

(Vgl. Section 6.)

Dr. K. L. Wolters (Dessau).

Nach Erlass der staatlichen Prüfungsvorschriften haben wir uns im Hygienischen Institut für Anhalt mit Verbesserung der Diphtherie-Impfstoffe zur aktiven Immunisierung beschäftigt. In vergleichenden Untersuchungen mit nativen Formoltoxoiden, Aluminium-Hydroxyd und Kali-Alaun-Präzipitat-Impfstoffen von gleichen Flockungseinheiten wurde die Antitoxinbildung beim Meerschweinchen nach der Methode von Jensen ausgewertet. Hierbei zeigten Kali-Alaun-Präzipitat-Impfstoffe 80fach, Aluminium-Hydroxyd-Impfstoffe 14fach stärkere antigene Wirkung als native Formoltoxoide von gleichem Flockungswert. Nach diesen Versuchen wurde vom Anhaltischen Serum-Institut Dessau ein Kali-Alaun-Präzipitat-Impfstoff produktionsmässig hergestellt und zur staatlichen Prüfung gegeben. Die Auswertung im Staatsinstitut ergab 95 Schutzeinheiten während die bisher geprüften Impfstoffe, native Formoltoxoide, im allgemeinen nur 1 Schutzeinheit im ccm. enthalten. Die Forderung des Reichsgesundheitsamtes nach Verbesserung der Diphtherie-Impfstoffe in Deutschland ist damit erfüllt. Durch Impfung von 1 ccm. dieses Kali-Alaun-Präzipitat-Impfstoffes wird ein ausreichender Impfschutz erzielt.

Prof. F. von Gröer (Lwów).

Pathergometrische Messungen an mit Diphtherie-toxin vergifteten Meerschweinchen (Versuche von *Korzynski*) und an gegen Diphtherie mit Toxoiden immunisierten Kindern (Versuche von *Ramert*) haben ergeben, dass grössere, einmalige Dosen des Diphtherietoxins bzw. Toxoids zuerst die Diphtherietoxinempfindlichkeit des Probanden *steigern* und erst später herabsetzen. Diese Steigerung der Diphtherietoxinempfindlichkeit kann durch die Anwendung hoher Dosen des Antigens bis zur *Anergie* getrieben werden. Diese Versuche werfen zum ersten Mal ein Licht auf das biologische Geschehen in Diphtherietoxinvergiftetem Organismus *vor* dem Auftreten des Antitoxins und mahnen zur Vorsicht bei der prophylactischen Immunisierung bei drohender Infektionsgelegenheit.

Dr. A. Schmidt-Burbach (Dessau).

Auf einen besonderen Vorteil der Präzipitat-Impfstoffe sei hier kurz hingewiesen, den ich im Verlaufe vergleichender Immunisierungsversuche feststellen konnte, nämlich auf die Möglichkeit der gleichzeitigen aktiven und passiven Schutzimfung. Mit nativen, unmodifizierten Toxoiden ist die Simultan-Impfung bekanntlich deswegen nicht möglich, weil das Toxoid durch das gleichzeitig eingespritzte Antitoxin sofort neutralisiert wird. Anders bei Präzipitat-Impfstoffen, insbesondere beim Alaun-toxoid ; es ist im subkutanen " Depot " an Alaun gebunden und dadurch für das Antitoxin unerreichbar. Nur allmählich wird das Toxoid aus dem Depot herausgeschwemmt und resorbiert. Die Neutralisation des Toxoids kann demzufolge auch nur allmählich und in kleinen Dosen erfolgen, wobei es jedoch stets kleine immunisatorische Reize auszuüben vermag. So entsteht bei der gleichzeitigen Einspritzung von Alauntoxoid und Serum eine Antitoxinkurve, die bis zum 6. Tage abfällt, dann etwa 2 Tage auf derselben Höhe bleibt, die stets *über* dem Mindestwert von 0,02 AE/ccm. liegt, und vom 8 Tage an, wieder aufsteigende Tendenz zeigt. Es ist demnach möglich, mit Hilfe eines Präzipitat-Impfstoffes (bei diesen Versuchen wurde ein Alauntoxoid des Anhaltischen Serum Instituts verwendet) und eines antitoxischen Serums, durch gleichzeitige aktivpassive Schutzimpfung eine *sofort eintretende und lange anhaltende* Immunität zu erreichen.

Prof. Th. Madsen (Copenhagen).

In Denmark, Drs. Schmidt and Hansen have prepared a highly purified formol-toxoid, which they have combined with aluminium hydroxide. One injection will immunize 90 per cent. of those injected. In order to obtain a higher immunity, Cl. Jensen has proposed to reinforce this single injection with instillation into the nose of a few drops of formol-toxoid 4, 5 and 6 weeks later.

B.—Whooping-Cough

Prof. W. H. Park (New York), **Opener**: *Pertussis Prophylactic Vaccination.*

An investigation was carried out to determine the value of pertussis vaccine in preventing pertussis infection. The problems in this investigation were (1) to test pertussis vaccines that were prepared by different methods; (2) to determine the optimum dosage for vaccination; (3) to study agglutinin response in vaccinated children and compare it with agglutinin response in clinical pertussis.

Groups of children were given different pertussis vaccines; some of them by intracutaneous, and others by intramuscular or subcutaneous injection. The dosage given varied between 1 and 88 thousand million bacilli. The agglutinin response was studied in several groups of vaccinated children some of whom received small and others large doses. Agglutinin studies were also made in clinical pertussis and in control children.

During February 1933—June 1936 a total of 2,707 children were vaccinated. The controls consisted of a group of 355 children who were injected with sterile milk instead of vaccine, and another group of 5,676 unvaccinated children.

The results are given in the following Table:—

PERTUSSIS PROPHYLACTIC VACCINATION

Total Vaccinations during 1933-1936 in two Institutions and two Clinics in New York City

Total Dosage (Thousand Millions)	Children on each Dose	Developed Pertussis				Incidence	
		Total Number	Period after Vaccination			Total	1–18 Months after Vac.
			During Vac.	Less than 1 Month	1–18 Months		
65–88	793	8	1	1	6*	1.01%	0.76%
50–60	604	16	4	2	10	2.65%	1.65%
30–49	666	11	1	2	8	1.65%	1.20%
16–29	222	12	1	6	5	5.40%	2.25%
1–15	422	26	9	4	13	6.16%	3.08%
Total Vaccinated	2707**	73	16	15	42	2.69%	1.55%
CONTROLS							
Total Number	6031***	429	16	22	391	7.11%	6.48%

* Three of the six cases that developed Pertussis 1 to 18 months after vaccination did not whoop or vomit, and the cough cleared up within 2 to 3 weeks.

** 112 of the vaccinated children could not be located and were therefore not followed up.

*** 115 of the control children could not be located and were therefore not followed up.

The agglutinin response in the vaccinated children was proportional to the dosage given, and the highest agglutinin titre was obtained 1 to 2 weeks after vaccination was completed. After that it went down rapidly, although in most instances it remained on a higher than normal level for quite some time. The agglutinin curve in the vaccinated children corresponded to that of clinical pertussis, although it did not attain the same height.

From the results obtained, so far, the following conclusions may be drawn :—

(1) The dosage for prophylactic vaccination should be approximately 65 to 88 thousand million bacilli. This is in accord with the recommendation of Sauer.

(2) Although the incidence of pertussis in New York City is comparatively low among the unvaccinated children, there was a marked reduction in the number of cases among the children that were vaccinated with 65 to 88 thousand million bacilli as compared with the unvaccinated controls.

Pertussis prophylactic vaccination with potent vaccines and adequate dosage seems desirable.

(3) There was apparently little difference in the efficacy of the different new whooping-cough vaccines that we tested, when similar dosage was used.

(4) These conclusions are only tentative as approximately half of the children in this investigation have been followed for less than one year.

Prof. Th. Madsen (Copenhagen) : *The Control of Whooping-Cough.*

Vaccination against whooping-cough, that much-debated subject, has continued to give good results in Denmark.

The method of making the vaccine is as follows :

Forty-eight-hour cultures of 15 to 20 different, freshly isolated whooping-cough strains are suspended in physiological saline containing 1 per cent. of a 40 per cent. solution of formaldehyde. The suspension stands from four to seven days at 37° C., is then centrifuged, the top fluid is thrown away, and the vaccine is again suspended in saline containing 0.5 per cent. phenol. Usually three injections are given intramuscularly or subcutaneously.

The difficulty of determining the effects of the whooping-cough vaccine lies in obtaining suitable material. The only material I know of is the record from the Faroe Islands, two epidemics in 1923 and 1929, the results of which are given below.

	3,926 Vaccinated	1,073 Non-vaccinated
Deaths	6	26
Percentage	0·15	2·4

These show that the vaccination has *some* prophylactic effect, but that its efficiency appears especially from the effects on the course

of the disease, as the number of severe cases and the mortality among those vaccinated were much lower than among those not vaccinated. It must be remembered that in these cases the vaccination was made one or two weeks before the wave of the epidemic came. We have no experience as to how long the immunity (increase of resistance) lasts. In Denmark we have extensive experience of the therapeutic effect of whooping-cough vaccine, and, according to reports from practitioners and health officers, there is almost universal agreement that, used early in the disease, it shortens its course and mitigates the symptoms.

In two cases there were deaths after the second injection on newborn infants. Whether there are special circumstances to be taken into consideration in the case of such infants, or phenomena which possibly may always be observable when injections are made, shall be left unsaid, but in Denmark vaccination in the first month of life is discouraged for that reason.

Dr. L. W. Sauer (Evanston, U.S.A.) [Communicated by Dr. A. D. Gardner (Oxford, England)]: *The Control of Whooping-Cough by means of* Hæmophilus pertussis *Vaccine.*

The bacteriological diagnostic laboratory isolates fresh strains from pertussis patients each month by the Chievitz-Meyer cough-plate technique. Each lot of vaccine is prepared from five to seven recently isolated, hæmolytic strains of *H. pertussis* (Bordet-Gengou). Since 1933, strains are checked for Phase I characteristics (Leslie-Gardner technique). The Dold test is performed with each strain—the intracutaneous injection of 0.1 c.c. of a 1,000 million suspension should cause necrosis of the rabbit's skin. All Bordet medium is enriched with 10 per cent. fresh, defibrinated human blood. The three-day growth (37° C.) is scraped off, killed in 0.5 per cent. phenolized physiological solution of sodium chloride, and diluted to approximately 10,000 million bacilli per c.cm. Except when in transit, it is kept at 2°–10° C. from the time it is scraped off until it is injected. In eight years (1928-36), about 2,400 selected non-immune children, from six weeks to five years of age (average about twelve months) have been injected. The total of 8 c.cm. is divided into three, bilateral, weekly injections. One, 1.5 and 1.5 c.cm. are injected just under the skin of each arm. Children over three years get 10 c.cm. (1.0, 2.0 and 2.0 c.cm. bilaterally). Syringe and needles are sterilized in the oven at 180° C. for one hour. From three and one-half months to seven years after injection, 143 children were intimately exposed to whooping-cough in non-injected (control) siblings in their households: 258 were casually exposed outside the household a total of 481 times. In all, sixteen failures have occurred, eight of them in four families. Five were in children over three years of age when injected. The disease was light in nine, typical in six, and severe in one. Potency (freshness) of strains, refrigeration of the vaccine, dosage, the interval between

injection and exposure, and age at the time of injection, seem to govern results. The immunity, activated by this antigen, simulates that which develops from the disease.

Dr. A. D. Gardner (Oxford, England): *Whooping-Cough*:—*Difficulties and Applications of the Cough-plate Method.*

Many bacteriologists meet with so little success when first attempting the bacteriological diagnosis of whooping-cough that the method is in constant danger of falling into discredit.

The chief requisites for success are skill and time. The examination of the plates must be in the hands of a bacteriologist or first-rate technician who has been instructed in the technique and is able to give ample time to it. Time, again, is necessary for the taking of the plates; overworked nurses in hospital wards cannot do the work properly. On the other hand, success in poor homes proves that no high degree of skill or intellect is needed. In average conditions 75 per cent. of positive results in the early stages may be expected, so that the early identification of an outbreak is certain.

The method has also been applied to the determination of the period of infectivity in individual patients and their consequent release from isolation. Kendrick and Elderling in 1935 were able by this means to release over half of their patients in the fourth week from the onset of the disease, and 90 per cent. before the end of the fifth.

In a warm-weather epidemic last spring and summer, Dr. R. E. Smith and I found that the majority of patients had ceased to expectorate *H. pertussis* by the middle of the fourth week, and by their early release a great economic saving was achieved. By following up the released patients it was proved that they were harmless to susceptible children.

Dr. N. Silverthorne (Toronto) [Communicated by Dr. N. E. McKinnon (Toronto)]: *The Control of Whooping-Cough.*

We have made a study of the cough-plate method of isolation of *H. pertussis*. Cough-plates were taken with great care in duplicate. Six hundred children have been examined in this way, of which 359 have had clinical whooping-cough. Our results showed 98 per cent. positive cough-plates in the first week of the disease.

A study of the morphological, cultural and serological characteristics was made of freshly-isolated and old stock strains. Our results showed that freshly isolated strains were a homologous series and that they varied in several respects from old stock strains.

In the last two years we have inoculated over 200 children with a fresh strain vaccine in an attempt to actively immunize them. No conclusions can be drawn as yet from this work, but the results are encouraging.

Dr. I. H. Maclean (London).

It is important that good technique should be employed in the preparation and exposure of plates. Increasing the concentration of blood in the culture medium to 50 per cent. has given me better results than formerly. As a further method of diagnosis *H. pertussis* vaccine may be injected into a patient and the agglutinins formed in response to this secondary stimulus can be demonstrated by the slide technique. Hæmolysis which has been recorded so frequently by American workers has not been obvious in my experience, and I should like further information on this point.

Dr. Lucy Mishulow (New York).

In reply to Dr. Maclean, hæmolysis is not observable round single colonies, but is evident when strains are subcultured on to plates containing rabbit blood.

Dr. Perrin H. Long (Baltimore, U.S.A.).

Our experience with pertussis vaccine has led us to the conclusion that further experimental investigations upon the essential immunizing fraction of the pertussis bacillus are needed. We have been unable to demonstrate antibacterial antibody in the sera of rabbits immunized by the intravenous route with standard American commercial vaccines. Our own vaccines likewise failed to produce immune sera. However, animals immunized with phase I living pertussis bacilli, produced good immune sera which have definite antibacterial power. This can easily be demonstrated by the prevention of the spread of a pertussis bacillus lesion in the skin of the rabbit.

Prof. Th. Madsen (Copenhagen).

In Denmark for the last 20 years the routine practice has been to release patients 5 weeks after the onset of the disease (4 weeks after the onset of the specific whoop). This decision has been made on the basis of our wide bacteriological experience, and the results have been very satisfactory.

Dr. J. V. Duhig (Queensland) and **Prof. H. Schmidt** (Marburg), also took part in the Discussion.

DISCUSSION *on* THE PROPHYLAXIS AND SERUM TREATMENT OF HUMAN AND ANIMAL DISEASES CAUSED BY ANAEROBIC BACTERIA

Chairman : Dr. R. A. O'Brien (Beckenham, England).

Mr. T. Dalling (Beckenham, England), **Opener**. [Communicated by Dr. H. J. Parish (Beckenham).]

In Britain, the anaerobes causing disease in domesticated animals are *Cl. tetani*, *Cl. chauvœi*, *Cl. œdematis maligni* (*Vibrion septique*),

Lorsque l'organisme humain (ou animal) peut encore réagir à l'injection d'un sérum spécifique, le succès de la sérothérapie dépend : (1) de la richesse en anticorps différents du sérum injecté, et (2) de la mise en jeu de la loi de la cataxie.

(1) La toxine microbienne est très complexe ; elle représente *un ensemble* d'antigènes nocifs pour l'organisme. L'exemple du *B. perfringens* le montre surabondamment.

Nous avons montré jadis, avec Mr. Nasta, que l'hémolysine de certaines souches de *B. perfringens* joue un rôle primordial, plus important que la toxine non-hémolytique dans l'intoxication causée par cet anaérobie. Depuis, il a été démontré que le *B. perfringens* sécrète d'autres toxines ; myotoxine, neurotoxine et toxine nécrosante, chacune de celles-ci pouvant avoir un rôle prédominant dans la constitution antigénique d'un type de *B. perfringens*.

Pour qu'un sérum thérapeutique ait le maximum d'efficacité, il faut donc qu'il renferme des anticorps vis-à-vis de tous les antigènes produits par une espèce microbienne.

Nous avons donné le nom de *holosérum* (sérum complet) à ce sérum pour le distinguer des sérums *polyvalents*, actifs vis-à-vis de plusieurs espèces microbiennes.

Voici résumées quelques données expérimentales qui justifient notre manière de voir sur la valeur du sérum antitoxique et antimicrobien (sérum anti-exo-endotoxique).

(*a*) Le sérum anti-*Vibrion septique* uniquement antitoxique neutralise (chez la souris) 1/20 ou 1/10 de cc. de la culture homologue ; le sérum à la fois antitoxique et fortement antimicrobien neutralise ½ cc., 1 cc., 1 cc. ½ et, quelquefois 2 cc. de la même culture.

(*b*) Les sérums à la fois anti-toxiques et anti-microbiens préparés par injection de microbes et de toxines (à quelques heures d'intervalle) ou d'anaculture, sont les plus actifs (Weinberg et Barotte).

(*c*) Le sérum anti-*Vibrion septique*, riche en antitoxine anti-*chauvoei* ne neutralise la culture de ce dernier anaérobie que lorsqu'il renferme, en même temps, une quantité notable d'hémolysine anti-*chauvoei*.

(*d*) Nos dernières recherches sur les sérums préparés vis-à-vis de différents types de *B. perfringens* apportent une preuve nouvelle qu'un sérum de titre antitoxique moyen, devient très efficace lorsque son titre antimicrobien est très élevé (Weinberg et Guillaumie).

(2) *La loi de la cataxie* (brisement de l'association microbienne par neutralisation de l'espèce dominante) que nous avons établie avec Ginsbourg pour les infections polymicrobiennes, joue également dans les cas d'infection causée par un seul microbe, lorsque le sérum utilisé renferme des anticorps très actifs contre la toxine dominante de l'espèce microbienne, cause de la maladie.

Dr. A. D. McEwen (Wye, England) : *The* Bacillus welchii *Toxæmias of Sheep.*

The *B. welchii* toxæmias of sheep are of alimentary origin, and none is caused by the classical type of *B. welchii*, but by bacteria

producing the classical type of *B. welchii* toxin and at least one other toxin.

Lamb dysentery and a disease of adult sheep, the latter caused by a bacillus first named *B. paludis*, have respectively a separate geographical distribution, indicating that a pathogenic distinction divides the causal micro-organisms of the two diseases. Nevertheless, certain strains of the "lamb dysentery bacillus" and of *B. paludis* appear to be toxicogenically similar.

A frequent feature, common to both diseases, is the ulceration of the small intestine; this lesion is not found in the entero-toxæmias caused by *B. ovitoxicus*. The "lamb dysentery bacillus" and *B. paludis* give rise to a potent necrotic toxin which is not produced by *B. ovitoxicus*, and it is possible that this toxin is the cause of ulceration. There is no proof, however, that the toxin is the primary cause of disease in sucking lambs or in adult sheep, and the epidemiology of the two diseases suggests that a different toxin may be primarily responsible for each.

The fractionation of culture filtrates of the "lamb dysentery bacillus" and of *B. paludis* has shown that while both micro-organisms may produce the same toxins, yet each bacillus probably produces one particular toxin in greater abundance than does the other. It is suggested that further light might be shed on the toxins primarily responsible for each disease by a detailed study of the toxins in intestinal filtrates from lambs and sheep.

The most important toxin of *B. ovitoxicus* is also produced in some degree by certain strains of the "lamb dysentery bacillus." The toxin is slowly elaborated in laboratory cultures and it is thermo-stable, but, when it is obtained from the intestinal contents of cases of entero-toxæmia or from cases of lamb dysentery, it appears to be thermo-labile and to have been very rapidly produced. These inconsistencies might be explained by the observation that toxin in the intestine is rendered more potent by an activator, which is also found in the filtrates of intestinal contents of normal sheep, this activator being thermo-labile.

The types of *B. welchii* causing toxæmia in sheep all occasion a typical gas gangrene infection on parenteral inoculation, and the possibility of their causing wound gas gangrene in man should be kept in view.

Prof. J. Russell Greig (Edinburgh). [Communicated by Dr. W. S. Gordon (Edinburgh)]: *The Prophylaxis of Braxy.*

Various forms of vaccine have been used.

Formalinized whole cultures, prepared from the *Vibrion septique*, have reduced the incidence of the disease in infected areas from 7 per cent. to 0.64 per cent. During the last four years, approximately 200,000 doses of vaccine have been issued by Moredun Institute, and the results have been consistently successful. A method of double vaccination, with an interval of fourteen days

between each inoculation, was originally practised, but more recently it has been found that a single inoculation affords adequate protection, except on certain farms on which the disease occurs in a very virulent form.

Dr. A. W. Turner (Melbourne). [Communicated by Dr. D. W. Henderson (Elstree, England)]: *Prophylaxis of Black Disease by Formolized Culture (Anaculture).*

Black Disease (infectious necrotic hepatitis) of sheep and cattle in Australia, Tasmania and New Zealand is a toxæmia following the germination of spores of *B. œdematiens* in foci of hepatic necrosis. The bacterial infection is initiated by the presence of a metazoan parasite, *Fasciola hepatica*, which acts by producing *loci minoris resistentiæ*. Hence prevention may be directed against the fluke, the anaerobic infection, or both.

Believing that effective immunity against *B. œdematiens* depends largely upon the antitoxin content of the blood, we introduced in 1928, a formolized vaccine (anaculture) prepared from cultures of maximum toxicity. The bacillary bodies were retained mostly for technical simplification and for their local effect in delaying absorption, and also because any "O" antibody produced might inhibit the proliferation of germinated spores; this possibility is now being investigated.

The vaccine protected guinea-pigs against 50 M.L.D. and sheep against 20 M.L.D. of highly toxic culture given intramuscularly.

In the field it has been used extensively in two doses of 5 ml. given at an interval of four weeks, combined with anti-fluke measures, which have themselves caused a great lowering of mortality.

Since 1931 the Commonwealth Serum Laboratories have issued vaccine for 1,107,800 sheep.

In five flocks examined by us, there were 197 deaths amongst 4,340 controls (4.54 per cent.) as against 69 amongst 4,532 vaccinated (1.52 per cent.). The New South Wales Department of Stock conducted a series of trials which included some sheep given three injections at monthly intervals. The results are shown in the Table :—

Season.	Total No. (divided into three groups).	Percentage of Losses.		
		Three injections of vaccine.	Two injections of vaccine.	Controls.
1931–32	2,630	0.9	1.9	6.03
1932–33	3,422	0.16	1.7	2.4
1933–34	6,448	0.36	1.6	3.4

For the period 1928-1934, sheep in properly controlled experiments of which records are available, totalled 34,967 ; there were 502 deaths out of 13,742 controls (3.65 per cent.), 219 out of 16,337 twice-vaccinated (1.34 per cent.) and 24 out of 4,888 thrice-vaccinated (0.49 per cent.). The value of prophylactic vaccination against black disease has thus been clearly demonstrated.

Mr. H. W. Bennetts (Western Australia). [Communicated by Dr. D. W. Henderson (Elstree, England)]: *The Control of Infectious Entero-Toxæmia of Sheep in Australia.*

Experimental data and field evidence indicate that active immunization with a formol-culture prepared from *Cl. welchii* (Type *ovitoxicum*) provides a safe, efficient and economical method of prophylaxis against infectious entero-toxæmia of sheep. The application of the method in the field presents two difficulties. First, the incidence of the disease is erratic and a high death-rate may result in newly-affected localities before preventive measures can be instituted. Secondly, the disease frequently occurs in lambs which are too young to be actively immunized ; the experience in Western Australia is, however, an exception in this respect.

It has not yet been determined whether the antitoxic immunity transmitted to the lamb through the colostrum is of a sufficiently high degree to protect against the natural infection.

There is some evidence that sheep in the original enzootic areas acquire some natural immunity to the disease and that animals in recently developed areas are relatively more susceptible. This evidence should be considered when making recommendations for the control of the disease.

An investigation of the factors predisposing to the multiplication and toxin production of *Cl. welchii* (Type *ovitoxicum*) in the contents of the small bowel of sheep may indicate methods of control which will supplement or replace the method of active immunization.

***Prof. I. M. Velicanov** (Moscow) : *La bactériologie et la sérothérapie de la gangrène gazeuse et du botulisme.*

La Gangrène Gazeuse.—L'analyse de 84 échantillons du sol, recueillis dans des localités différentes de l'U.R.S.S., a montré la présence de *B. perfringens* dans > 95 pour cent.

B. perfringens et *B. œdematiens* jouent le rôle principal dans le développement de la gangrène gazeuse. Le traitement avec un mélange des sérums spécifiques a eu un succès invariable (trois morts sur 64 cas).

L'application prophylactique des sérums a un succès moindre que la sérothérapie.

Le Botulisme.—Dans le travail de laboratoire quotidien nous nous servons du milieu que nous avons élaboré—celui de soya et de fèves—pour la culture de *B. botulinus.*

La culture dans des conditions particulièrement favorables nous permet d'obtenir une toxine forte dont la dose léthale minime pour le cobaye varie de 0.00001 à 0.0000001 cc.

Les chevaux, immunisés par l'anatoxine, donnent comme résultat des sérums antibotuliques d'un haut titre.

Nos observations confirment la possibilité d'une période d'incubation très brève pour le botulisme.

Le botulisme non-traité par le sérum donne une mortalité très haute (93 pour cent). L'application du sérum spécifique réduit fortement la mortalité (18 pour cent).

Dans les cas de l'introduction tardive du sérum et de l'intoxication progressant rapidement, l'effet curatif du sérum est possible à condition de son introduction à grandes doses.

L'immunisation répétée par l'anatoxine confère à l'homme une immunité.

Prof. J. P. Scott (Philadelphia). [Communicated by Dr. L. D. Bushnell (Manhattan, Kansas)]: *Prophylaxis in Gas Gangrene of Animals.*

In a report to the 12th International Veterinary Congress, A. W. Turner, L. R. Vawter and myself suggested that these diseases might be classified as follows:—

(1) *Specific diseases.* Blackleg, Bacillary hæmoglobinuria and Black disease.

(2) *Diseases of the intestinal tract.* (*a*) Bacterial: Braxy, etc., (*b*) Toxæmias: Pulpy kidney disease.

(3) *Wound infections.* Malignant œdema, etc.

In Kansas, anti-blackleg serum has cured about 65 per cent. of affected cattle. Formolized blackleg filtrates, and bacterins prepared from cultures of *Cl. chauvœi*, have reduced losses from blackleg to less than 1 in 20,000.

Blackleg in sheep has been controlled by the use of either *Cl. chauvœi* or *Cl. œdematis maligni* filtrates, depending on the organism isolated. Blackleg in swine was controlled in three cases by repairing fences, etc., which had caused skin lesions and thus allowed the entrance of *Cl. œdematis maligni.*

Precipitation of blackleg filtrates and bacterins by potassium alum increased the immunizing properties of these agents, but did not greatly increase their aggressive activities. The precipitate was found to contain almost all the immunizing substances; the supernatant fluid was of little or no value.

Malignant œdema of horses and cattle has been treated chiefly by means of surgical drainage and the use of chemicals.

Dr. G. Ramon (Garches). [Communicated by Prof. M. Weinburg (Paris)]: *La prophylaxie du tétanos chez l'homme et chez les animaux domestiques au moyen de l'anatoxine tétanique.*

C'est par une série de travaux poursuivis à partir de 1923 que nous avons établi la méthode de prophylaxie du tétanos, chez

l'homme et chez les animaux domestiques, au moyen de l'anatoxine tétanique.

Dès l'abord des recherches effectuées chez l'homme adulte avec la collaboration de Ch. Zoeller nous ont permis (*a*) de déterminer les conditions de la vaccination antitétanique; (*b*) de préciser le nombre, le volume, l'espacement des doses d'anatoxine tétanique; (*c*) de mettre en évidence le rôle de l'injection dite " de rappel " qui fait rebondir l'immunité; (*d*) de conférer au nouveau-né l'immunité antitétanique en vaccinant la mère (avec Nattan, Larrier et Grasset); et enfin, (*e*) de montrer la possibilité de pratiquer simultanément la sérothérapie et la vaccination antitétanique.

Un grand nombre de travaux sont venus, peu à peu, confirmer la valeur de cette méthode d'immunisation antitétanique active et ont de plus affirmé l'intérêt qu'elle présente pour la prophylaxie du tétanos chez les individus et dans certaines collectivités particulièrement exposées.

Depuis plusieurs années, la méthode de vaccination contre le tétanos à l'aide de l'anatoxine spécifique a fait son entrée dans la pratique. En France, elle est réalisée surtout sous la forme de vaccinations associées (G. Ramon et Ch. Zoeller).

Dans des essais préliminaires effectués parallèlement à ceux réalisés chez l'homme nous avons avec la collaboration de P. Descombey jeté, dès 1924, les bases de la prophylaxie du tétanos chez les animaux domestiques et en particulier chez le cheval, au moyen de l'anatoxine tétanique.

Au cours de ces essais, il a été principalement établi que (*a*) l'immunité procurée, par exemple, par deux doses d'anatoxine tétanique (de valeur antigène suffisante) de 10 cc. chacune, injectées à un mois d'intervalle, met le cheval à l'abri de l'infection tétanique expérimentale qui provoque chez l'animal non vacciné un tétanos rapidement mortel; (*b*) l'immunité antitétanique due à l'anatoxine spécifique peut-être notablement accrue grâce à l'addition à cet antigène d'une substance telle que le tapioca, par exemple, qui provoque une inflammation locale au point d'injection (1924); (*c*) l'immunité que confère la vaccination anatoxique est considérablement renforcée par une seule injection d'anatoxine dite " de rappel," faite dans un délai plus ou moins éloigné de la vaccination.

Ces principes ont permis d'obtenir chez le cheval des sérums antitétaniques de haute valeur antitoxique.

Appliquée sur grande échelle en France, la méthode a maintenant fourni les preuves de son efficacité (G. Ramon et E. Lemétayer).

Dr. Z. Mikhailova and **Dr. I. Jatzevitch** (Moscow): *L'immunité passive et active contre le tétanos chez l'homme.*

Le sérum humain normal ne contient aucune antitoxine spécifique contre le tétanos.

Les analyses systématiques du sang humain à intervalles différents après l'introduction prophylactique du sérum antitétanique à la

dose de 3,000 unités antitoxiques internationales indiquent le terme bref de la présence d'antitoxine dans leurs sérums (5-11 jours).

L'immunisation par l'anatoxine tétanique (3 cc.) faite à deux reprises à intervalle de 15-20 jours confère une immunité élevée.

L'application d'une double prophylaxie contre le tétanos—introduction du sérum et de l'anatoxine—donne de meilleurs résultats. Quand les injections simultanées du sérum antitétanique et de l'anatoxine sont faites à deux ou à trois reprises, l'immunité active est conférée plus rapidement que dans le cas d'immunisation par l'anatoxine seule.

La création d'un " dépôt " dans l'organisme, à l'aide d'une huile végétale (anatoxine + huile) confère aux hommes une immunité active, encore plus rapide et plus stable.

Le perfectionnement de la qualité de l'anatoxine nous permettra de nous borner à une seule injection, ce qui facilitera considérablement la réalisation de la prophylaxie contre le tétanos.

Dr. K. L. Wolters (Dessau) : *Über aktive Immunisierung gegen Tetanus.*

Die grundlegenden Arbeiten von Ramon über die immunisierenden Eigenschaften des mit Formol entgifteten Tetanustoxoids veranlassten uns, Versuche mit nativen Formoltoxoiden zur aktiven Immunisierung anzustellen. Wir haben festgestellt, dass es gelingt mit einmaliger Impfung von 1–2 ccm. Tetanus-Formoltoxoid Meerschweinchen gegen die 50fach tödliche Giftdosis zu schützen ; durch zweimalige Impfung sind Meerschweinchen gegen die 500fach tödliche Giftdosis für mehrere Monate geschützt. Wir haben demzufolge in Deutschland in grösserem Umfange Formoltoxoide zur Immunisierung bei Pferden mit Erfolg verwendet. Für Impfungen beim Menschen halten wir gereinigte Toxoide für zweckmässiger. Durch Dialyse und Ultrafiltration rein dargestellte Tetanustoxoide haben die gleichen immunisierenden Eigenschaften wie ungereinigte native Toxoide. Immunisierung von Meerschweinchen mit gereinigten Kali-Alaun-Präzipitat-Toxoiden (in ähnlicher Weise wie Kali-Alaun-Präzipitat-Toxoide bei Diphtherie nach Glenny gewonnen) ergab bei Impfung von 0.25—1 c.cm. nach 5 Wochen Schutz gegen die 500–5,000fach tödliche Toxindosis. Die Überlegenheit der gereinigten Kali-Alaun-Präzipitat-Toxoide gegenüber nativen Formol-Toxoiden ist so gross, dass wir zur aktiven Immunisierung gegen Tetanus bei Mensch und Tier nur gereinigte Kali-Alaun-Toxoide verwenden werden. Über Herstellung, Antitoxinbestimmung, und die Erfahrungen bei gleichzeitig aktiv-passiver Immunisierung wird ausführlich im Zentralblatt für Bakteriologie berichtet.

Dr. A. Stroe (Bucarest). [Communicated by Prof. M. Weinberg (Paris)] : *Angine gangréneuse compliquant la diphtérie. Traitement par le sérum anti-gangréneux.*

Sur 1,135 diphtériques soignés a l'hôpital entre 1927–34 on a

isolé 115 cas de diphtérie maligne par l'association de la diphtérie avec l'angine de Henoch.

Les frottis directs démontrent l'existence d'une flore abondante formée, en dehors de cocci et bacilles diphtériques, de diverses espèces de bacilles gramophiles ou Gram négatifs.

Quelques uns de ces bacilles sont gros, taillés droit, gramophiles, d'autres minces, sinueux, Gram négatifs, plus longs que les bacilles diphtériques et qui se disposent en groupes de 6 à 8 bacilles, enfin un coccobacille Gram négatif, ressemblant à celui de Pfeiffer. Dans les milieux anaérobies poussent différentes formes de bacilles comme *Cl. perfringens*, *Cl. œdematiens* et un coccobacille, donnant des colonies, visibles à la loupe, et qui ne pousse que dans les milieux strictement anaérobies avec du sang.

Le traitement au sérum anti-gangréneux, est fort encourageant. Dans les formes où la flore Gram négatif prédomine, cette sérothérapie ne donne pas les résultats escomptés.

Par l'isolement de ces multiples espèces de microbes et en examinant leurs toxines, on pourrait peut-être arriver à une préparation de sérums, qui mélangée à la méthode de Weinberg, peut donner de bons résultats dans les différentes épidémies, variables justement par ce polymorphisme microbien.

Dr. J. Celarek (Warsaw) : *The Treatment of Gangrenous Diphtheria with Gas Gangrene Antisera.*

Severe cases of diphtheria showing gangrene of the throat have been treated with a mixture of diphtheria and gas-gangrene antitoxins. This treatment was initiated by Professor Szenejch in Warsaw two years ago. The results have been satisfactory. Some cases have been examined for anaerobes by Dr. Feygen and myself, and, while we have been unable to find *B. perfringens* or other organisms of this group, we have found an anaerobic staphylococcus in some cases. Our study of the staphylococcus hæmolysins, which can be neutralized to some extent by gas gangrene antisera, may, perhaps, explain the good results obtained in the treatment of gangrenous diphtheria with these sera.

Mr. A. T. Glenny (Beckenham, England).

It is surprising that the blood of many normal horses which we have examined has neutralized *Cl. welchii* δ toxin that is produced by Wilsdon type C strains.

Dr. R. A. O'Brien (the Chairman) and **Prof. M. Weinberg**, also took part in the Discussion.

DISCUSSION *on* THE PROPHYLAXIS AND SERUM TREATMENT OF PNEUMONIA

Chairman : Prof. W. H. Park (New York).

Dr. J. G. M. Bullowa *(New York),* ***Opener.***

Ninety-five per cent. of the endemic pneumonias in adults are due to various types of pneumococci. The types occur in varying proportion from year to year and from month to month. At present at Harlem Hospital less than 1 per cent. of pneumococci isolated from adults cannot be classified in the 32 types of Cooper. The present position of serum therapy is due to improvements in methods for the prompt and precise classification of the responsible organism and to improved therapeutic sera and methods of administration.

The determination of pneumococcus type may be made in 76 per cent. of the cases by a direct Neufeld examination of the sputum. Where too few organisms are present, it may be necessary to propagate them in white mice, where they are usually found in the peritoneal exudate after 3 to 4 hours, or in the mouse blood after 8 to 10 hours. The organism obtained from the sputum is responsible for the pneumonia in 93 per cent. of the cases, as shown by comparison with simultaneous or subsequent lung aspiration and blood culture.

A routine blood culture should be taken to determine the presence of bacteræmia, which does not always occur at the onset of the pneumonia. Most of the complications of pneumonia as well as the deaths are associated with bacteræmia and may be prevented by the adequate administration of serum. Thus large doses should be given early in the disease and the most potent serum should be used, as this gives more protection in a shorter time. With severe cases we endeavour to give the entire required dose at once, sometimes in a single intravenous drip, which dilutes the antibody, thus preventing reaction. Frequently, after a single dose of serum, there is a permanent fall of pulse and temperature, and specific agglutinins appear in the blood. The agglutinins in the blood serve as a "marker," e.g., when there is very strong agglutination it is unnecessary to give additional serum, and if the pulse and temperature remain elevated another explanation must be sought. There may be invasion with an additional or a different type from that for which serum was given, a complication or serum sickness.

The skin response to specific soluble substance (Francis) in cases with adequate protective antibody is valuable corroborative evidence, but should not be relied upon as the sole test of adequate treatment.

For purposes of comparison of serum treated and non-serum treated cases, we now regard as adequately treated, patients who receive 100,000 units in 24 hours, if non-bacteræmic, or 200,000 units in 24 hours if bacteræmic, or a lesser dose if a previously negative agglutination test becomes positive. It is found that with serum,

the mortality rate is reduced and termination of the disease is earlier. This is especially well-shown with Type I cases, where the mortality without serum is more than 25 per cent. and with serum less than 10 per cent. For Type II the reduction in mortality is not so marked, but is still significant, and becomes more noticeable when the cases are treated before the fourth day. Because in some cases the blood is invaded quite late in the disease, it is probably wise to treat even late cases with serum in the hope that blood invasion may be prevented. The influence of serum on mortality and in shortening illness is also shown for Types IV, V, VII and VIII.

The experiment fostered by the Commonwealth Fund in Massachusetts has shown the value of serum in the hands of general practitioners. Nine hundred cases were treated by five hundred physicians and the mortality was reduced by half.

We still need :—

(1) The production of stronger, cheaper and more refined sera ;

(2) the production of serum from different animals. We have already employed refined therapeutic Type I rabbit serum without reactions. This is necessary so that cases may be treated who are sensitized to horse serum ;

(3) better and more reliable methods for the control of dosage, in the interest of efficiency and economy.

The prophylaxis of the pneumonias is a subject of the greatest current interest. The approach may have to be by means of immunization against the common cold, or influenza viruses, which prepare for the subsequent bacterial invasion. Eighty per cent. of my cases have a history of a head or chest cold preceding the chill or chest pain which marks the onset of pneumonia.

Acknowledgment is made to the Littauer Pneumonia Research Fund, Harlem Hospital, and the Metropolitan Life Insurance Company, New York City.

Dr. R. L. Cecil (New York) : *Analysis of a Series of Cases of Lobar Pneumonia in which Serum was administered very early in the Disease.*

When patients with pneumococcal pneumonia are treated with homologous serum during the first 24 to 48 hours of the disease, the following phenomena are frequently observed :

(1) The disease may be completely aborted, the temperature and the pulse and respiration rate dropping to normal within 12 to 24 hours after the administration of serum.

(2) Striking improvement in the patient's general condition, due to the disappearance of toxæmia.

(3) Early serum treatment prevents the spread of infection from one lung to another, and even limits the area of infection in the lobe primarily infected.

(4) Bacteræmia is prevented or, if already present, is quickly checked.

(5) The leucocytes rapidly return to normal.

(6) Homologous agglutinins, precipitins and protective bodies promptly make their appearance in the circulating blood.

(7) Skin tests become positive to the homologous polysaccharide.

(8) *The death-rate is cut one-half to two-thirds in the types for* which serum has been employed.

(9) These conclusions are derived chiefly from studies on Type I and Type II pneumonia, though evidence is rapidly accumulating that they apply with equal validity to several other types as well.

Dr. L. D. Felton (Baltimore, U.S.A.): *Possibility of Prophylaxis against Pneumococcus Infections.*

Three experiments have been carried out during the past three years. In each, the group consisted of a fluctuating population of young men averaging about 23 years of age. In the first experiment, 3,000 men were immunized by a single injection of 2 mgs. each of Type I and Type II soluble antigen (an active form of the polysaccharide of Heidelberger and Avery). A group of 9,000 men under the same conditions constituted a control for the study. A sample of the immunized group, 180 in all, was tested for antibody production by titration of the serum before and after immunization. Protective antibody, varying in amount from one protective unit per c.c. of serum to 0.01 unit, was found in all one month after inoculation, in 97 per cent. three months after, and in 65 per cent. of 25 tested one year later. The period of observation was from December 1933 to April 1934. Whereas in the control of 9,000 there were 8 cases of pneumonia with 2 deaths, no cases occurred in the inoculated group of 3,000.

In the second experiment, 14,000 young men were inoculated and 14,000 used as controls. One mg. each of Type I and Type II antigen was injected instead of 2 mgs. as in the previous experiment. This reduction in amount was necessary because of the severe reaction following the injection of a 2 mgs. dose. The period of observation was from November, 1934, to June 15, 1935. In the 14,000 inoculated men there were 13 cases of pneumonia with no deaths, and in the control group, 23 cases with 2 deaths. It is fair to say that in the inoculated group, 5 of the cases were recorded as mild or abortive, while in the uninoculated group no mild cases were reported.

The third experiment, in which some 30,000 men are inoculated, is being conducted this year.

Dr. R. Cruickshank (London): *Biological Differences among Types I, II and III Pneumococci: their Relationship to Lobar Pneumonia and its Treatment with Serum.*

The serological differentiation of the pneumococcus types depends on qualitative differences in the chemical structure of the capsule which is probably identical with the specific soluble substance (S.S.S.) of the organism. The virulence of the pneumococcus is associated with the capsular or specific soluble substance, although there is no convincing evidence that the latter is in itself toxic. The present communication attempts to establish certain differences (other than serological) between types I, II and III pneumococci, associated mainly with *quantitative* differences in the capsular substance or S.S.S. The thesis is that type I pneumococcus has less capsular substance than type II, and type II less than type III, and that the severity of pneumococcal infections, particularly lobar pneumonia, varies *directly* with the amount of capsular substance or S.S.S. elaborated by the infecting pneumococcus.

Differences in the morphology, colonial appearance, production of S.S.S. *in vitro* and in experimental and human infections, and in the antigenicity of pneumococcus types I, II and III were demonstrated and correlated with differences in the severity of lobar pneumonia caused by these three types. Another factor in the infectivity of the pneumococcus is its invasiveness, which seems to vary *inversely* with the amount of capsular substance. The relationship of these differences to the serum therapy of lobar pneumonia was discussed.

Dr. G. J. Langley (Manchester): *Lobar Pneumonia—The Agglutinin Control of Serum Treatment.*

During the years 1934 to 1936, serum treatment has been in use at Hope Hospital, Salford, but has been strictly limited to cases between the ages of 16 and 60 who were admitted before the end of the third day of the illness. Of 330 cases admitted, 100 received serum treatment. When it is possible to determine the type of pneumococcus quickly, 40,000 units of homologous serum are usually given intravenously. When immediate typing is not possible, that is, during the night or when the result is in doubt, 20,000 units of each of sera, Types I and II, are injected, the homologous serum being used on succeeding days. Serum is given daily, always intravenously, till agglutinins appear in the patient's blood. The serum used in all cases has been a concentrated globulin, the agglutinin titre of which has been estimated. The time that elapses between the administration of serum and the test for agglutinins has never been less than four hours.

It would appear from the figures that serum treatment in pneumonia yields good results when the dosage is controlled by the agglutination test, but this method cannot entirely replace control

by clinical judgment and experience. It does, however, minimize the amount of serum necessary and reduces the cost of treatment. If the temperature chart be relied upon and serum administered until crisis has occurred, a greater quantity of serum will be utilized, and the already high cost still further increased. In non-serum treated cases, agglutinins usually appear some hours before the crisis.

The main difficulty appears to lie in maintaining a trustworthy capsulated, agglutinable bacterial emulsion, a matter reasonably simple in a large and specialized service, but difficult elsewhere.

The mortality rate for Type I pneumonia was 20.5 per cent. in cases treated without serum, and 7.7 per cent. in serum treated cases; for Type II it was 39 per cent. in cases treated without serum, and 10 per cent. in serum treated cases.

Dr. Lois Stent (Manchester): *The Control of Dosage in the Serum Treatment of Pneumonia.*

A microscopic agglutination test was used in this investigation; it differed from that used by Sabin (1930) in the fact that the agglutination was observed in a wet preparation and no staining was used. The emulsions of pneumococci were made from organisms grown on a solid medium.

The titre of the serum used in treatment was sufficiently high for agglutinins to be detected in the patient's serum after 20,000 units had been given, and gave a higher reading with the microscopic agglutination test than the usual method.

In the practical application of the test, the blood serum from patients was examined for the presence of agglutinins 12-18 hours after the administration of 20,000 units of Type I or Type II anti-pneumococcal serum; if typing was delayed, a combined dose of 20,000 units of each serum was given. Further serum was withheld if there was a marked agglutination reaction, but repeated when the reaction was moderate or weak.

Approximately, 100 cases of Type I and Type II pneumonia have been treated in this way, and the results of the agglutination tests are given.

Dr. L. E. H. Whitby (London): *Laboratory Control of Pneumonia.*

Serum therapy is not justified in pneumonia unless the type has been established—this partly because of expense and partly because of serum reactions.

Reliable methods for typing from sputum are by the direct method and by modifications involving staining, by mouse inoculation and by agglutination of a culture.

With the direct method, errors in typing may be due to lack of experience (much is required), failure of the typing serum to penetrate the specimen (hence an erroneous Group IV result), stale

sputum or to paucity of cocci in the specimen. With the direct method the capsular change is different with each specific type; the Type III capsule also shows non-specific, but easily recognized capsular swelling with heterologous serum. Mouse inoculation should confirm all direct tests especially with a Group IV result; the method only fails with avirulent or dead cocci which do not multiply in the mouse.

Typing by injecting therapeutic serum and observing absorption of the specific agglutinin is unreliable; the agglutinin present in a patient's serum after injection depends more on the titre of the serum injected than on absorption of a specific agglutinin by the patient.

Judgment as to whether small numbers of pneumococci in sputum indicate pneumococcal pneumonia, depends on clinical data; a lobar distribution of the lesion is significant. In influenzal periods or in sputum containing *Hæmophilus influenzæ*, a pneumonia is not often pneumococcal.

For estimating the sufficiency of serum dosage the clinical features are the most reliable index. Assessment of resistance by agglutinin content of the blood is unreliable. Agglutinins appear to develop normally at or about the crisis, but when developed and even persistent are no criterion for regarding a patient as safe from relapse, extension or complication. Passively induced agglutinins, in high titre, are not always associated with an immediate crisis. When agglutinins disappear, in conjunction with clinical improvement and a normal leucocyte count, resolution is complete and the infective process overcome.

Positive blood cultures in the early stages are usual; a persistently positive blood culture is of grave significance.

The leucocyte count is of value for prognosis and for indicating complications; the count is raised by serum sickness.

Type I infections occur in the relatively young, and the prognosis, even without serum, is better than in all type infections. Serum need only be given to those Type I subjects in whom the prognosis is anxious. Type II infections are very toxic and all should receive serum. In a series of 184 cases of lobar pneumonia it was concluded that Type I and Type II serum was of proved value and should be given to all cases, even late in the disease, where it was felt necessary to exploit every remedy to achieve cure.

Dr. D. G. Leys (Birmingham): *The Serum Treatment of Pneumonia.*

Although anti-pneumococcus serum has been available for many years, its use appears to be confined to a few centres and individuals. Technique is difficult, and discredit has been brought by underdosage, delay in administration and the use of routes other than intravenous. It is very important to establish a minimum standard of dosage.

Efforts to control dosage by means of estimates of the patient's immunity response are commendable, but they imply a technique which demands skilled assistants; they are inapplicable to private practice and to many hospitals in Great Britain. Blood culture is valuable, but results are slow and not sufficiently informative to provide the sole guide to dosage. Clinical estimation of the prognosis is notoriously fallacious.

An attempt has been made to use the sedimentation rate of red blood cells as a prognostic guide. The technique is simple and rapid, and the results of trials are encouraging. The fall is greatly delayed during the acute phase of the disease and until death occurs in fatal cases; it does not return to normal with the critical fall of temperature, but shows an emphatic change towards the normal. When this change has occurred, it has indicated ultimate recovery, although alteration in the opposite direction may again occur with the development of empyema. Illustrative charts were demonstrated.

Two cases of brachial neuritis, as a definite complication of a mild serum fever, have occurred in a series of 133 patients treated with Type I antiserum. Both were young men, and neither case was bacteræmic. There was some residual weakness in both cases. The incidence (1.5 per cent.) appears to be much greater than the reported cases would suggest to be usual in patients treated with other antiserums.

Nutrition is the most important single factor in determining attack rate and mortality in pneumonia; no other single factor could possibly account for the tremendous class difference in incidence and mortality.

Prof. F. G. Blake (Yale Univ., U.S.A.): *The Serum Treatment of Pneumonia.*

Experience with serum therapy of pneumonia indicates that early treatment is essential. The necessity for early treatment may be predicated upon the fact that lobar pneumonia is a rapidly progressive infection, a true emergency, which, like diphtheria, permits of no delay if cure is to be accomplished. The crucial period is the first 48 to 72 hours. During this period the local pulmonary inflammation, beginning ordinarily as a unilobar lymphangitis, quickly followed by outpouring of inflammatory œdema into the alveoli, passes on rapidly to hepatization. It seems probable that immune serum can only enter and affect the local infection during this period of congestion and serious exudation. Accompanying, and arising from, the pulmonary infection are three rapidly progressing general manifestations—bacteræmia, anoxæmia and "toxæmia." The first two are measurable, the third hypothetical and not understood. While the relative importance of these phenomena in determining a fatal issue is difficult to

unravel, their injurious effects on vital functions, including humoral and cellular defence, are manifest. Since humoral defence is the only one supplied by antiserum, it seems probable that it must be provided before cellular defence is so impaired that it can no longer co-operate in bringing about recovery.

Dr. Thomas Francis, Jr. (New York) : *The Intracutaneous Test as a Guide to Serum Therapy.*

A positive reaction of the immediate wheal and erythema variety occurs only in the presence of circulating type specific antibodies, and indicates a physiological capacity of the tissue cells to respond to the stimulus of the S.S.S. which is injected. The absence of either antibody concentration or cellular activity results in a negative test.

Eighty-seven cases of Type I pneumonia were treated with serum ; 78 recovered and 9 died. In 76 of the 78 cases which recovered, the test often became positive before the temperature became normal ; the remaining 2 cases, in which characteristic positive tests were not obtained, were a negro with empyema and a patient with active rheumatoid arthritis, in which absorption from the skin was long delayed. On the other hand, in 9 fatal cases, no positive reactions were obtained, although considerable circulating antitoxin had been demonstrated in several instances.

It is extremely important to use highly purified material and a careful technique of injection.

We have also carried out skin tests with the somatic or " C " polysaccharide of the pneumococcus (first described in collaboration with Tillett), precipitins to which appear in the serum promptly with the onset of illness and disappear in convalescence. The skin reaction with the " C " substance differs from that with S.S.S. in its gross appearance. An immediate flare and wheal may be observed which is followed by a delayed brownish red œdematous reaction surrounded by a halo. In cases which recover, skin tests are positive throughout the acute illness and negative after recovery ; in fatal cases, although precipitins continue in the serum, the skin reactions may be either negative throughout, or positive early in the disease, but negative on subsequent tests. This failure of fatal cases to react, or alternatively, the loss of a pre-existing activity, may prove to be of prognostic value. Furthermore, the lack of cutaneous response in the presence of antibodies is similar to that occurring with the S.S.S. in fatal cases.

Considered as a biological test which may present certain, but infrequent, irregularities, the skin test with S.S.S., and possibly also with " C " substance, has proved to be extremely valuable as a guide to serum therapy. As with all other tests, however, its value is enhanced when accompanied by good judgment. When in doubt give more serum !

Prof. A. R. Dochez (New York) :

In view of the progress reported in the treatment of lobar pneumonia by specific antisera, I think that emphasis should be directed to this form of treatment. There, is, however, another factor which plays a possible rôle in healing. It has been shown that there exist in the exudate of lobar pneumonia certain unsaturated fatty acids which are powerful bactericidal agents, and are particularly effective against the pneumococcus, killing the organism, *in vitro*, in dilutions of from 1 in 30,000 to 1 in 200,000. It would seem probable that they may play some part in local healing in the lung. They are probably derived from lecithins set free from disintegrated leucocytes and subsequently acted upon by lipase, which liberates unsaturated fatty acids in the form of the sodium soap. This phenomenon seems worthy of further investigation, since by increasing the available supplies of these fatty acids, the healing process might possibly be made more effective.

Prof. L. S. P. Davidson (Aberdeen), also took part in the Discussion.

DISCUSSION *on* THE RELATIVE VALUE OF ANTITOXIC AND ANTIBACTERIAL IMMUNITY IN THE PROPHYLAXIS AND TREATMENT OF HUMAN AND ANIMAL DISEASES IN WHICH THE INVASION BY THE CAUSATIVE BACTERIUM MAY OCCUR IN A FOCAL OR GENERALIZED FORM

Chairman : Prof. W. H. Park (New York).

Dr. A. B. Wadsworth (Albany, U.S.A.), **Opener :** *Antitoxic and Antibacterial Sera in the Treatment of Infection.*

Effective serum therapy depends primarily upon the neutralization of toxic substances ; secondarily, upon the destruction of the bacterial incitant. The extent to which one process is dependent upon the other, as well as the precise sequence of events in the different infectious diseases, is a most important field for investigation. Unfolding knowledge of group- and type-specificity reveals the subtle interrelationships between the antitoxic and antibacterial activities of serum and the protective mechanism of the tissues of the host that underlie effective serum therapy.

The antitoxic action in serum therapy has been contrasted very sharply with the antibacterial phenomena attributed to the sera that are used in the specific treatment of infectious diseases, the bacterial incitants of which invade the tissues generally. So sharply have the two activities been differentiated, that the antitoxic action of these sera has been overshadowed. In the laboratory

study of the different activities or manifestations of immune sera, it is important to classify and differentiate sharply, but when one turns to the study of effective serum therapy in the infectious processes of the tissues, it is not only difficult but often impossible to distinguish these activities.

Antitoxic serum exerts an antibacterial effect, possibly directly, but more obviously indirectly, upon the incitant of infection in the tissues. Diphtheria antitoxin definitely and specifically neutralizes the toxæmia of diphtheria. Recovery is often critical and complete, but when incomplete the parasitism of the diphtheria bacillus is harmless to the host. There is a direct antitoxic effect which destroys the toxin and prevents injury of the tissues ; and an antibacterial effect, since the protective mechanism of the tissues is conserved and the diphtheria bacillus destroyed. In these reactions there is no evidence of type-specificity.

The antibacterial sera exert an antitoxic effect either directly or indirectly upon the incitants of the more generalized infectious diseases. Antipneumococcus serum has a definite specific action, sharply defined according to type. The antibacterial action is demonstrated by the protection test in mice and by the effect of the serum on phagocytosis ; the antitoxic action is manifest in the crisis induced by the serum. The neutralization of the toxin may precede or follow the destruction or inhibition of the pneumococci in the tissues. However, following neutralization of its toxic activity, the pneumococcus may persist as a parasite relatively harmless to the host, as evidenced by its survival in man after the crisis of pneumonia, and in the immunized horse when the organism becomes attenuated and its toxic activity nil.

Antimeningococcus serum, similarly, is manifestly directly antitoxic and antibacterial in character. Neutralization of the toxic activity may be titrated and the antibacterial effect determined by the protection test.

In the treatment of infection clinically, and experimentally in laboratory studies, anti-streptococcus serum has an antitoxic and an antibacterial action which is complicated by intricate subtle group- or type-specificities of the toxic and antigenic activities of the different hæmolytic streptococci. The antitoxic as well as the antibacterial activities are manifestly essential to effective serum therapy. In the earliest stages of scarlet fever and erysipelas, the antitoxic action may suffice as it does in diphtheria ; but in streptococcus infection, homologous relations between the sera and the incitants are important.

Although, in general, the neutralization of the toxin takes place in the homologous relation according to groups, the potency and valency of the different antitoxic sera vary widely. Combinations of representative sera may broaden the valency beyond that manifest by either serum separately. Finally, and what is of the utmost importance, qualities may be supplied by the tissues of the

animal host, which doubtless account in large measure for the manifestations of the antitoxic as well as the antibacterial effect of heterologous sera in infectious processes whether studied clinically in the treatment of human infections or experimentally in animals.

Prof. L. Cotoni and **Dr. J. Pochon** (Paris) : *Les méthodes générales d'immunisation dans le traitement des infections streptococciques.*

L'immunité antistreptococcique, malgré des recherches anciennes et nombreuses, demeure imparfaitement connue. D'une façon générale les streptocoques apparaissent jusqu'ici doués d'un médiocre pouvoir antigéne vis-à-vis des animaux d'expérience. Il est difficile de vacciner la souris et la vaccination du lapin échoue souvent par les méthodes qui réussissent chez d'autres espèces microbiennes. Cependant l'emploi de cultures totales additionnées de ricinoléate de sodium, cultures administrées à doses faibles et répétées plusieurs jours de suite, permettent la vaccination du lapin par voie veineuse. L'étude expérimentale de l'immunité est limitée par la rareté des échantillons de streptocoque tuant le lapin à faibles doses, capables d'être utilisés pour l'épreuve. Mais il n'est pas douteux qu'on peut apporter la preuve expérimentale de l'immunité pour certains streptocoques d'origine équine (gourme) ou aviaire (épizooties de poules).

On peut à l'aide des mêmes échantillons, obtenir des sérums doués de pouvoir préventif et curatif contre la septicémie expérimentale du lapin. L'action croisée des sérums vis-à-vis d'échantillons étrangers de streptocoque est problématique. La part de l'immunité antitoxique dans l'immunité antistreptococcique est difficile à préciser.

En pratique, *bactériothérapie* et *vaccination préventive* forment deux chapitres à peine ébauchés ; seule la *vaccination antiscarlatineuse* a reçu une large application. La *sérothérapie* est encore peu avancée, l'émiettement du streptocoque en races antigènes multiples demeurant une grosse difficulté.

Quel est l'avenir de pareilles recherches ? On peut espérer du progrès de la classification des streptocoques réalisé depuis quelques années, une connaissance plus précise de l'étiologie et de l'épidémiologie des infections streptococciques. L'étude des toxines pourra faciliter la préparation de sérums antitoxiques plus puissants et peut-être doués d'un domaine antitoxique plus étendu. D'autre part ce serait un progrès important de prévoir le pouvoir antigène d'un échantillon de streptocoque d'après l'aspect de ses colonies et de n'utiliser exclusivement pour l'immunisation que des souches particulièrement antigènes. Un autre progrès serait l'acquisition d'une méthode précise permettent le titrage de tous les sérums antistreptococciques. En présence des difficultés rencontrées dans le titrage chez l'animal, il y a lieu de poursuivre l'étude des méthodes *in vitro*, comme nous avons tenté de le faire pour le pneumocoque et différentes espéces microbiennes par la neutralisation des anticorps

in vitro. Des progrès dans la concentration des sérums seraient également désirables. En matière de sérothérapie on ne saurait dissimuler que beaucoup d'obstacles encombrent encore la route qui mènera au but.

Dr. H. J. Parish (Beckenham, England) : *Antitoxic and Antibacterial Immunity in Hæmolytic Streptococcal Infections.*

Hæmolytic streptococci may attack by (*a*) soluble toxins and (*b*) invasion of tissues. A relatively heat-stable ("erythrogenic" or "Dick") toxin predominates in scarlet fever ; and invasiveness in puerperal fever and erysipelas. There is no specificity of strains in relation to a particular infection.

Antitoxic Immunity. Diluted erythrogenic toxin may be used to indicate immunity to scarlet fever, but not to other streptococcal diseases. Antitoxic serum is of prophylactic and therapeutic value in scarlet fever—neutralizes toxin and to some extent prevents complications ; does not influence established complications ; is of limited usefulness in erysipelas, puerperal septicæmia, etc., in which the erythrogenic toxin and antitoxin are of importance only occasionally. In rabbits, antitoxin neutralizes the erythrogenic toxin, but does not prevent "invasion" of joints if culture is injected. In mice and rabbits, the protection conferred against virulent "septic" strains is of low order.

Active immunization with toxin reduces incidence of scarlet fever in (*a*) hospitals and (*b*) communities. There is no evidence that tonsillitis replaces scarlet fever in immunized subjects. Nor does immunization of nurses in fever hospitals appear to increase the risk of conveyance of scarlet fever to non-scarlatinal patients.

Streptolysin (a thermolabile toxin) and fibrinolysin (the soluble enzyme or toxin described by Tillett and Garner) may play some part in the rapid generalization of infections ; both appear to be antigenic.

Antibacterial immunity. The streptococcidal action of the blood may be increased in streptococcal infections or may remain stationary. When it increases it is presumably a response of the immunity mechanism of the host to the infection, although an enhanced power of killing streptococci cannot be correlated with recovery.

In man, the bactericidal power of the whole blood may be influenced by the injection of bacterial suspensions, as Sir Almroth Wright and his collaborators have shown. The increase in resistance is regarded by many as too slight and too temporary to be of practical therapeutic value.

The use of so-called "antibacterial" sera is theoretically desirable, but the preparation and testing of such products are beset with difficulties. The "antibacterial" sera issued commercially have been obtained from horses injected repeatedly with several strains of streptococci, and are virtually devoid of antimicrobial action

Because of variation from strain to strain, it would appear to be necessary to employ for treatment an antiserum corresponding to the strain causing the infection. With the facilities available at present, this is quite impracticable.

Chemotherapy may to some extent replace serotherapy in many hæmolytic streptococcal infections. Recently "Prontosil" and *p*-Aminobenzenesulphonamide have been shown to be of value in the treatment of hæmolytic streptococcal infections in the laboratory animal and in man.

Dr. R. Cruickshank (London): *Antitoxic Immunity in Relation to Streptococcal Infection.*

Immunization with streptococcal toxin has not been widely adopted for various reasons; the difficulty of preparing an effective non-toxic antigenic mixture, the mildness of scarlet fever and the fact that immunization apparently protects only against the scarlatinal rash. This latter fact was demonstrated in the immunization of the Nursing Staff of Glasgow Royal Infirmary during the past eight years. While there was a diminution of, but not complete, protection against scarlet fever, streptococcal tonsillitis remained a prevalent and intractable infection. Analysis of our results showed that tonsillitis was almost as frequent in naturally Dick-negative nurses as in those immunized or non-immunized. In these circumstances, wholesale immunization against scarlet fever does not seem a desirable procedure except for administrative reasons, e.g., among nursing staffs and in children's institutions.

However, there is some indication from an analysis of complications following scarlet fever among children at different age-groups that an antitoxic immunity may help to lessen the incidence of these complications, i.e., may inhibit the *invasiveness* of the streptococcus without preventing infection with that organism. Support for this view was found in the incidence of streptococcal puerperal infection among Dick positive and Dick negative parturient women. If such is the case, then immunization of children with streptococcal toxin would be worth while.

Dr. L. Colebrook (London): *The Possibility of Doing Harm by Means of Anti-Streptococcal Sera in Infections other than Scarlet Fever.*

The efficacy of anti-streptococcal sera in infections *other than scarlet fever* is still in dispute among clinicians although these sera have been in use all over the world for 40 years. This is largely due to the fact that most of the data have been collected from experience with puerperal fever cases—at least 50 per cent. of which are not infected with hæmolytic streptococci.

An analysis of all the hæmolytic streptococcus-infected cases of puerperal fever admitted to Queen Charlotte's Hospital (where

serum is not used) during 5 years, has shown that, whereas the death-rate for all such cases (431) who received no serum at any time was 19 per cent., the death-rate for 55 cases which had been given serum *before admission to the Hospital*, was 45.5 per cent. Considerations already published[1] seem to indicate that this great difference in mortality rates cannot be attributed to selection of particularly severe cases for serum treatment, although that may possibly have played a small part.

The increased death-rate in these human cases treated by anti-streptococcal sera, taken in conjunction with the experimental results in mice reported by Penfold and Butler[2] and also the well-known toxic effects of horse serum in the human subject, suggest that the injection of this serum in some patients may actually favour the development of a transient localized uterine infection into a much more lethal peritoneal, or septicæmic, infection.

The administration of anti-streptococcal sera in infections other than scarlet fever ought to be discontinued until sera are available which can be shown to exert a potent curative effect in animals infected some hours before with hæmolytic streptococci—and, therefore, more or less comparable with infected human patients.

No such effects have been demonstrated with any sera on the market at the present time.

Dr. Stuart Mudd (Philadelphia): *On the Use of Convalescent Serum in Streptococcal Infections.*

Several papers this morning have demonstrated the usefulness of antitoxin in treating scarlatina. Nevertheless, there is a considerable reluctance in our country to use horse antitoxin in scarlatina because of the frequency of serum sickness and unwillingness to sensitize unnecessarily to horse serum. The whole blood and serum of scarlatina convalescents used as a prophylactic or therapeutic agent is free from these objections. Gordon and collaborators from the Detroit, and Hayne, Levinson and Thalhimer[3] from the Chicago human serum centres have reported series of scarlatina cases successfully treated with pooled convalescent serum. Such serum has been used satisfactorily also in other types of streptococcal sepsis.[4]

In Philadelphia we have striven particularly to obtain pools from as many convalescent donors as possible in order to increase the statistical probability of having antibacterial antibody, as well as antitoxin, corresponding to the infecting type. Serum immediately on removal from the clot is frozen in a bath of solid CO_2 in a suitable suspending medium, and held frozen in a cold storage vault until the blood from 30 to 60 donors has been collected. The serum is.

[1] Colebrook, *Lancet*, 1935, I, 1085.
[2] *Med. Jour. Australia*, 1932, I, 717.
[3] *J. Amer. Med. Assoc.*, 1935, 105, 783.
[4] *J. Amer. Med. Assoc.*, 1935, 105, 864.

then thawed, pooled and filtered. In Philadelphia it is distributed after drying from the frozen state. Our results have been most encouraging, both in prophylaxis and treatment of scarlatina, and in treatment of other streptococcal sepsis.

Prof. J. Forssman (Lund): *Immunity against the Staphylococcus.*

As immunity against staphylococci can be conveyed to new, untreated animals by sera from immunized animals, it must to an essential degree depend on antibodies. The development curves shown by these antibodies indicate, however, that we have to do with antibodies of a hitherto unknown kind.

The fight against a staphylococcal infection, both in actively and passively immunized animals, is a slow process. In rabbits which have acquired such a degree of immunity that they survive the intravenous injection of a large dose of staphylococci, the organisms do not reappear in the blood after their first disappearance. However, the weight of the animals falls by 10 to 20 per cent. according to the degree of immunity during the first 5 to 15 days after the infection, when a change takes place in which the weight remains constant and then increases. *This change occurs at about the same time as the disappearance of the staphylococci from the organs.*

In the organs, the staphylococci remain longest in kidneys and heart, except in some cases where they are to be found very late in the bone-marrow of the vertebræ, giving cold abscesses which soon compress the spinal cord and cause paralysis of the legs.

With reference to the slowness in combating the staphylococci in immunized animals, this immunity furnishes an analogy to immunity against anthrax and is in opposition to our experience with streptococci and pneumococci.

Immunity against the staphylococcus can be produced experimentally in several ways, of which I have studied two, viz., injections of (*a*) formolized staphylococcal suspensions, and (*b*) formolized, filtered broth cultures. Immunity was produced by both methods, but in varying degrees, dependent partly on the culture employed and partly on the varying individuality of the test animals. The degree of immunity obtained has been measured by the ability of the animal to survive a dose of staphylococci which is certainly fatal for normal animals.

(*a*) *Immunization with formolized staphylococci.* In one series of rabbits immunized with a particular strain, immunity was not reached until about a month after the completion of immunization, but lasted unchanged for a further period of 51 days. In a subsequent series of immunizations using other strains, the same degree of immunity was reached in 17 days. This immunity was subsequently lost to some degree, so that animals succumbed to a fatal dose of staphylococci, but those that were not tested till later (60th to 83rd day) were resistant to a fatal dose; thus, after the climax on the 17th day the immunity decreased to a minimum, increasing again

to its former level, without any further measures being taken. On the 160th day all evidence of immunity had disappeared. Agglutinins, opsonins and antihæmolysins could not be demonstrated in the later period of immunity which must, therefore, be explained by some other factor.

(*b*) *Immunization with filtered, formolized broth cultures.* Here the first climax occurred earlier, viz. on the 9th to 14th days, otherwise the curve of immunity agreed closely with the previous series. As the original broth cultures contained large quantities of lysin and a certain amount was allowed to remain after formolization, antihæmolysin was developed by the immune animals. The climax of antilysin corresponded to the early climax of immunity, but the antilysin had either been reduced to a minimum or had disappeared at the later climax of immunity ; thus, no part is played by the antilysin, even in this method of immunization.

Sera from immune animals, at the maximum of immunity produced by either method, were capable of protecting new, untreated animals. The immunity produced must thus, to an essential degree, depend on antibodies. The development curve shown by these antibodies indicates, however, that they are of a hitherto unknown kind.

Prof. C. E. Dolman (Vancouver) [Communicated by Dr. N. E. McKinnon (Toronto)] : *Antitoxic and Antibacterial Immunity in Staphylococcal Infections.*

We have to compare, on the one hand, the efficacy of killed staphylococcus vaccines and staphylococcus toxoids in the prophylaxis and treatment of *localized* staphylococcal infections ; and on the other hand, the value of agglutinating antisera and that of antitoxic sera in the treatment of *generalized* staphylococcal infections.

In vaccine therapy it is assumed that injections of bacterial antigen raise the opsonic index and so lead to more effective phagocytosis. Thus, although the clinical types of staphylococcal infection may be attributable to specific soluble toxins rather than to specific bacterial substances, these highly pathogenic antigens are to all intents ignored by the vaccine therapist.

My colleagues, Drs. Kitching and Farrell, report (in the press) that mice and rabbits—having acquired a high titre of circulating antitoxin as a result of either a single injection of antitoxic serum or a series of injections of toxoid—showed complete protection against lethal doses of living staphylococci. A formalinized vaccine, containing the strain used in the preparation of the toxoid, failed to confer protection under parallel conditions ; while a mixture of toxoid and vaccine was not as efficacious as was toxoid alone.

Results reported from Canada and other countries have demonstrated the superior efficacy of toxoid therapy in the treatment of certain intractable staphylococcal infections.

Patients with countless boils often show an average, or a low, antitoxic titre, indicating that the body may derive no significant antigenic stimuli from such lesions. Injection of toxoid into healthy subcutaneous or intramuscular tissues brings about an increased antitoxic titre, and some measure of clinical immunity. This immunity is only *relative* and will depend on many factors including the *frequency and degree of exposure to contact with staphylococci*. Hence the special difficulty often experienced with patients who carry large numbers of toxigenic staphylococci on their nasal mucous membrane.

The anti-necrotizing and antileucocidal powers of a serum following toxoid immunization are perhaps more reliable criteria of clinical immunity than antihæmolytic titre. Neutralization of necro-toxin and leucocidin are far more significant as defence mechanisms against focal staphylococcal invasion than an increased opsonin content of the serum induced by administration of a vaccine.

Much work yet remains to be done on the type and number of strains which should be used in the preparation of toxoids but the injection of formalinized filtrates does induce a marked increase in specific circulating antitoxin and confers some measure of clinical immunity. Antistaphylococcal agglutinating sera never have been deemed efficacious, but adequate dosage of staphylococcus antitoxin has proved of great value when administered early.

Despite the many still outstanding problems it seems fair to state that, in staphylococcal infection, whether of focal or generalized type, the manifold soluble toxic metabolites of this micro-organism, rather than its cellular skeleton, determine its invasive powers; and further that, since the former substances are antigenic, these should be used in all their complexity for the provocation of antitoxic immunity.

Dr. J. E. Blair (New York): *Rationale of Toxoid Therapy in Staphylococcal Infections, with Reference to Serologic Grouping of Staphylococci.*

The use of staphylococcal toxoid in the therapy of staphylococcal infections has been reported by several investigators to be of definite value. It is reasonable to assume that toxoid therapy would be of benefit only in those infections due to toxigenic staphylococci. The greatest benefit appears to be obtained in the treatment of superficial staphylococcal infections. No apparent clinical improvement which could be attributed to the effect of toxoid was observed in a group of 38 patients with chronic staphylococcal osteomyelitis who were given a course of staphylococcal toxoid therapy by our associates.

By means of reciprocal agglutinin absorption tests we have been able to separate staphylococci from various sources into several

distinct serologic groups, which differ in their toxigenic capacities, and possibly in other manifestations of pathogenicity. Several strains from cases of osteomyelitis are placed in those groups which do not produce exotoxin.

It is suggested that the choice of antitoxic therapy, or of active immunization by means of toxoid, in any given case may be guided by the type of staphylococcus ætiologically responsible. It is further suggested that failure to obtain beneficial results with staphylococcal toxoid therapy may be due in part to the fact that the staphylococcus in question produces its pathologic effect by means of factors other than exotoxin ; antitoxin, or immunization with toxoid, in such a case would have no direct effect on the bacteria.

Prof. A. Fleming and **Dr. I. H. Maclean** (London) : *On the Use of Staphylococcus Antitoxin, Toxin, Toxoid and Vaccine.*

Antitoxic serum was administered to patients suffering from severe staphylococcal infections. The antitoxic content of the patients' serum was tested before and at intervals after the administration of the antitoxin. It was noticed that in many cases the rise was very slight compared with the number of units of antitoxin given. The results seemed to be the same, whether or not the infecting staphylococcus was powerfully toxigenic.

A large number of patients suffering from various localized staphylococcal infections were treated with toxoid or with toxoid and vaccine, and the results were compared with those obtained with vaccine alone, in similar cases, during the last twenty years or more.

The antitoxic response to toxoid, toxoid-vaccine mixtures, and toxoid-antitoxin floccules seemed to be about equal. The average antitoxic content of patients' serum before treatment was less than 1 unit per cc., and after treatment was 6 units. In most cases the antitoxic response was rapid and reached its maximum after the administration of a small amount of toxoid. The administration of further toxoid (in some cases large amounts) did not raise the titre.

Recurrences have been noted in a number of cases in which the antitoxic content of the blood was high.

It is claimed that treatment intended to raise the antitoxic power of the blood is insufficient for the effective treatment of staphylococcal lesions. Probably the best treatment is a combination of toxoid and vaccine.

The antitoxic response was found to vary greatly in different patients ; in some, the administration of a few units of toxoid was followed by a rise of antitoxin to 5 units or more per cc., while in others this figure could not be reached even when much larger amounts were given. In some cases recurrences occurred in patients with a high antitoxic content.

Drs. G. Ramon (Garches), **R. Richou** and **M. Djourichitch** (Belgrade) [Communicated by Dr. M. Djourichitch]: *Sur le mécanisme de l'immunité conférée par l'anatoxine staphylococcique à l'égard de l'infection par le staphylocoque virulent. Démonstration expérimentale.*

La résistance des animaux vaccinés par l'anastaphylotoxine vis-à-vis de l'infection provoquée par l'injection intraveineuse de staphylocoques apparaît bien comme étant sous la dépendance de l'immunité antitoxique caractérisée par l'antitoxine spécifique dont l'anatoxine a engendré la formation. D'autres facteurs groupés sous l'appellation de défenses naturelles, par exemple la phagocytose, etc. . . . interviennent sans doute mais sous l'égide de l'immunité antitoxique.

L'immunité antitoxique, atteint-elle un degré élevé chez les lapins vaccinés par l'anatoxine, la plupart d'entre eux résistent parfaitement à l'infection. N'est-elle que peu prononcée ou s'est-elle affaiblie au cours du temps, les animaux n'offrent qu'une résistance relative : ils ont une survie plus ou moins prolongée mais finissent par succomber. Est-elle absente comme chez les animaux vaccinés par exemple à l'aide du vaccin staphylococcique microbien, leur résistance est nulle ; elle n'excède pas celle des témoins non vaccinés.

L'ensemble de nos expériences et de leurs résultats affirme donc le rôle primordial de l'immunité antitoxique, et de l'antitoxine spécifique qui en est l'agent, dans la résistance à l'égard de l'infection expérimentale par le staphylocoque virulent des organismes vaccinés au moyen de l'anatoxine staphylococcique.

Miss M. Llewellyn Smith (London): *Circulating Antitoxin and Resistance to Staphylococcus Culture in Rabbits.*

Twenty-nine rabbits were immunized with a variety of staphylococcus antigens: vaccine, washed living cocci, toxoid, alum toxoid and T.A.F. Their antitoxin titres were determined and they were then given 8 average lethal doses of washed culture, intravenously. The rabbits were grouped according to their antitoxin titres immediately before the culture was given and regardless of the type of antigen used in their immunization. In the group with less than 1 unit of antitoxin, the mortality rate, as a result of the culture injection, was 80 per cent. The mortality rates in the groups containing between 1 and 8 units and more than 8 units respectively, were 25 and 9 per cent. When the results of individual rabbits are considered, it appears that there is a striking correlation between antitoxin and resistance to culture. This is not incompatible with Forssman's finding, since Forssman's toxins contain a hæmolysin for rabbits' erythrocytes which is different from the α toxin and not neutralized by α antitoxin.

In a few rabbits (4/13) which had survived the injection of culture, late paralysis of the hind limbs developed. This was traceable to a staphylococcal abscess in the spine and appeared to be unaffected by the presence of antitoxin.

Dr. W. A. Timmerman (Utrecht, Holland): *Relation between Staphylococcus Antitoxic Content of Serum and Resistance to Culture.*

Seventeen rabbits, with less than 0.05 units of staphylococcus antitoxin per cc. of serum were immunized with staphylococcus toxoid in two subcutaneous injections. Three weeks after the second injection the antitoxin content of the serum was determined; 3 rabbits had less than 1 unit per cc.; 6 from 1 to 5, and 8 more than 5 units. The 17 rabbits and 4 controls were injected subcutaneously with 1 cc. of a 24-hour broth culture of staphylococcus. The controls died in 1 to 3 days. All animals with less than 1 unit also died. Of 6 animals with 1 to 5 units, 2 died (33 per cent.) and of 8 rabbits with more than 5 units, 1 died (12.5 per cent.).

This experiment shows that the amount of staphylococcus antitoxin present in the serum is a good indicator of the resistance to living culture of staphylococcus.

***Dr. D. S. Murray** (London): *Staphylococcus Toxoid.*

Since publishing some results over a year ago[1] the speaker has continued to use Staphylococcus Toxoid in a large number and wide variety of cases. The favourable results already reported have continued, but the relapse rate over a longer period is fairly high. Most cases, however, respond to a second course. After using a variety of antigens it appears that a combination of an autogenous vaccine and staphylococcus toxoid in graduated doses gives a more satisfactory result.

Prof. A. Pettersson (Lund): *Les Anticorps Protecteurs contre les microbes sensibles à la bactériolysine.*

Par des recherches antérieures il avait été demontré que les leucocytes constituent l'agent bactéricide de l'animal contre plusieurs microbes influencés par les bactériolysines. Le staphylocoque ressemble beaucoup au bacille du charbon en ce qui concerne l'infection et l'immunité. Les leucocytes constituent la défense de l'animal contre tous les deux. La différence entre les deux microbes est que le leucocyte ne détruit le staphylocoque qu'après l'avoir englobé. Les phagocytes abordent et avalent les staphylocoques avirulents et il se forme par là un abcès. Le staphylocoque virulent tient par contre les cellules à l'écart, grâce à sa substance négative-

[1] *Lancet*, 1935, 1, 303.

ment chimiotactique, il s'établit seulement un œdème et l'infection fait des progrès. Mais si l'on introduit au cobaye des staphylocoques virulents avec une dose suffisante de leucocytes de cet animal, l'infection est arrêtée. La provocation d'un afflux de leucocytes à la place d'infection est une des actions de l'immunsérum antistaphylococcique. Si l'on ajoute à des staphylocoques virulents une quantité suffisante d'immunsérum, si l'on incube le mélange pendant deux heures à +37° et l'injecte sous la peau d'un cobaye de 250 g., il se produit à la place de l'injection en un jour un amas de leucocytes à noyau polymorphe. L'injection de la même quantité de staphylocoques traités avec du sérum normal ne fait naître qu'un œdème transparent.

Le bacille du rouget du porc se comporte à l'égard de l'immunité comme le staphylocoque.

Pour le pneumocoque la bactériotropine est l'anticorps le plus important produit par l'immunisation. C'est depuis le début de la phagocytose des organismes que l'aspect de l'infection change.

Dr. Muriel Robertson (London): *Illustrations from the Spore-bearing Anaerobes of Protection against Infection by Antibacterial Mechanisms.*

The examples used are *B. œdematis maligni* and *B. chauvœi*. In the first of these organisms Felix and Robertson (1928) showed that the antigen complex contained, in addition to the soluble toxin, the two bacterial components, " H " heat-labile and " O " heat-stable. Passive protection was established in mice against infection with spore calcium suspensions by the injection of a serum containing only antibodies to the " O " element. Active immunity was produced in guinea-pigs by vaccination with a purely " O " antigen by Henderson in 1934.

The *in vivo* action of a highly protective serum obtained by using formolized centrifuged suspensions of *B. œdematis maligni* as antigen, was described. The reasons for considering this to be called forth by a bacterial antigen other than " O " were given. This serum, it was shown, did not owe its action to the minimal amount of antitoxin present.

In antibacterial immunity, whether active or passive, it is the actual invasion that is primarily checked by an attack upon the bacillary bodies themselves. In many anaerobes, notably *B. œdematis maligni*, *B. novyi* and *B. welchii*, the proliferation is accompanied by a local production of toxin which spreads and acts as an activator for further growth by direct tissue injury and paralysis of phagocytosis at the foci of infection.

Antibacterial sera have no detoxicating effect, but are powerful deterrents to invasion. Antitoxic sera, which are incidentally never quite free from antibacterial elements owing to the fact that the toxins, used as antigen, contain lyzed bacteria and bacterial

antigens, are in general, detoxicating agents, and have only a secondary action on proliferation.

Dr. D. W. Henderson (Elstree, England) : *The Protective Substances in Specific Antibacterial Sera which Control Experimental Infections with* Cl. œdematis maligni.

A serum prepared by immunizing rabbits with washed and formolized suspension of *Cl. œdematis maligni* has been shown by the author to possess two kinds of antibacterial protective substances. One of these, the " O " antibody, has been adequately described. The nature of the second antibody is the subject of this communication.

The serum produced in this way was absorbed with homologous bacterial suspensions, variously treated, and the absorbed sera were tested in mice for residual protective antibodies using the intracutaneous technique previously described. The absorbed sera were also tested for " H " and " O " agglutinins.

The results may be summarized as follows :—

(1) The antitoxin content of the serum was measured and the amount present (<0.5 unit) was shown to play no part in tests. Even twenty units of antitoxin failed to give any protection against the chosen test dose.

(2) Serum absorbed with a washed and formolized suspension was found to have lost its protective value.

(3) Absorption with a steamed suspension (" O " antigen) reduced the " O " agglutinin titre to zero but left the " H " titre unaltered. The protective value was reduced by approximately 50 per cent.

(4) Treatment of a suspension with ethyl alcohol destroyed the " H " antigen. Absorption of the original serum with a suspension so treated completely removed the " O " antibody ; the " H " antibody was, however, only slightly reduced. The protective value was approximately 50 per cent. lower than that of the unabsorbed serum and it was, therefore, comparable with that of the serum from which the " O " antibody had been removed.

The somatic antigens of S. *typhi*, including the one believed to be associated with virulence, are unaffected by treatment with alcohol (Felix). Thus, if the conditions are analogous, a suspension of *Cl. œdematis maligni* so treated should remove from a homologous immune serum all of the protective antibodies related to the soma. The results presented in this communication indicate, however, that a potent protective element remains unabsorbed. On the other hand, simultaneously with the removal of both somatic and flagellar antibodies by absorption with formolized suspension, all of the protective value is lost.

It remains to be shown whether an immune-serum which is devoid of " H " antibody, and which has been prepared by using an

alcohol-treated suspension of *Cl. œdematis maligni*, possesses the two protective antibodies that are now known to exist. If, under these conditions, the " O " antibody alone is found to be present it must be concluded: (*a*) that treatment with alcohol destroys an, as yet, hypothetical somatic substance or (*b*) that the " H " antibody plays an important rôle in the serum prophylaxis of an experimental infection with *Cl. œdematis maligni*.

Dr. J. H. Mason (Onderstepoort, South Africa) [Communicated by Dr. H. J. Parish (Beckenham, England)]: *The Toxin of* Cl. chauvœi.

A toxin has been demonstrated in filtrates of *Cl. chauvœi* cultures. The intradermal injection of filtrate into guinea-pigs has proved to be the best method of demonstrating it, particularly if adrenalin is incorporated. This toxin is neutralizable by homologous antitoxin, by *Cl. œdematis maligni* antitoxin, and to a slight extent by the sera of some normal rabbits and bovines.

Two antigens are involved in the production of immunity to infection with *Cl. chauvœi*, viz., (1) the heat-labile toxin or its atoxic modification (toxoid) contained in culture filtrates and formol-cultures, and (2) a heat-stable antigen contained in filtrates, formol-cultures and suspensions of boiled bacilli.

There is no correlation between the amount of circulating antitoxin in an animal and the power of the animal to resist living culture, but there is a correlation between the amount of circulating antitoxin and resistance to toxin, injected intravenously or intradermally. The evidence indicates that the heat-stable antigen is the more important in the production of immunity to infection with *Cl. chauvœi* culture.

***Prof. A. Wolff-Eisner** (Berlin): *Beitrag zur Tetanus-Diskussion.*

In der antitoxischen Serum-Therapie laufen die theoretische Erwartung und der tatsächliche Heilerfolg nicht parallel. Wird aber der Tierversuch den praktischen Verhältnissen entsprechend angestellt, vermindert sich der Widerspruch.

Aus meinen Versuchen ergibt sich, dass die Toxin-Antitoxin-Bindung im Tierkörper kein reines Reagenzglas-Phänomen, sondern ein ausgesprochen biologischer Vorgang ist. Beim Tetanus erfolgte die Prüfung mit meiner Methode der subkutanen dosierten Erd-Infektion (Münch. med. W., 1926, Nr. 30). Während beim Menschen die prophylaktische Injektion von Tetanus-Antitoxin Schutz gegen Tetanus-Erkrankung verleiht, ist dies bei der Maus und beim Meerschwein nicht der Fall. *Folgerung*: Beim Tetanus versagt die prophylaktische Antitoxin-Wirkung bei verschiedenen Tierspecies. Die Toxin-Neutralisierung ist somit im Tierkörper ein komplexerer Vorgang, als es der Ehrlichschen Lehre entspricht.

Die Dönitzschen Versuche (intravenöse-Tetanus-Toxin- und danach intravenöse Antitoxin-Anwendung im Tierversuch) stützen nur bedingt die heute bei Tetanus und Diphtherie verbreitete Anwendung von Riesen-Antitoxin Dosen. Die Dönitzschen Versuche sind (an den Verhältnissen der Praxis beim Tetanus beurteilt) nach zu kurzer Zeit abgebrochen und müssten nach unseren Feststellungen über die Differenz der Antitoxin-Wirkung bei verschiedenen Tierspezies erneut angestellt werden. Weiter ist noch zu berücksichtigen, dass die intravenöse Tetanus-Toxin-Zufuhr weniger wirkungsvoll ist als die der Praxis mehr entsprechende subkutane.

Nicht nur beim Tetanus ist die Toxin-Antitoxin-Bindung komplexer Natur und offenbar biologisch gesteuert. Versuche, deren Beendigung die Macht der Verhältnisse verhinderte, zeigten analoge Ergebnisse auch beim Diphtherie-Toxin, wo Mitwirkung von Bakterien völlig auszuschliessen ist.

Weitere Ergebnisse: Schwere klinische Krankheits-Erscheinungen treten auf, wenn die Toxine zur Bindung an lebenswichtigen Organen gelangen. Befinden sich bindende Gruppen (Receptoren im Sinne Ehrlichs) an weniger lebenswichtigen Stellen, so binden sie das Toxin und hindern es, an lebenswichtigen Stellen zur Wirkung zu gelangen (Winkelried-Funktion). Das Gift bewirkt hier nur lokale Störungen, die unter Umständen unterschwellig bleiben.

Die Aufmerksamkeit der Fachgenossen möchte ich noch auf meine Befunde hinweisen, die beim Tetanus auf eine Trennbarkeit der Krampf-Wirkung und der Tod-Wirkung hinweissen. (*Centralblatt f. Bakteriologie, Band* 106, *Pfeiffer-Festschrift.*)

DISCUSSION *on* THE RELATIVE VALUE OF ANTITOXIC AND ANTIBACTERIAL IMMUNITY IN THE PROPHYLAXIS AND TREATMENT OF HUMAN AND ANIMAL DISEASES IN WHICH THE INVASION BY THE CAUSATIVE BACTERIUM MAY OCCUR IN A FOCAL OF GENERALIZED FORM

Second Day.

Chairman : Dr. R. A. O'Brien (Beckenham, England).

Dr. F. Gerlach (Mödling bei Wien), **Opener :** *Ueber Erfolge, Unzulänglichkeiten und Misserfolge der wichtigsten Schutz und Heilimpfungen gegen tierische Infektionskrankheiten.*

Abgesehen davon, dass der Wert verschiedener Immunisierungsmethoden verschieden zu beurteilen sein wird, je nachdem ob es sich um passive, aktive oder Simultanmethoden handelt, wird die Bedeutung solcher Impfungen hauptsächlich dadurch beeinträchtigt, dass oftmals ein nicht ausreichender oder nicht lange genug dauernder Impfschutz erzielt wird. Ein gutes Immunisierungsverfahren muss einfach und gefahrlos anzuwenden und von sicherer und

dauerhafter Wirkung sein. In diesem Zusammenhange muss daran erinnert werden, dass im Gefolge von Impfungen mit lebenden Krankheitserregern Keimträger und Dauerausscheider entstehen können.

Die Dosierung lebender Krankheitserreger für Schutzimpfungen ist am schwierigsten. Sie gelingt leichter bei abgeschwächten und abgetöteten Bakterien, am besten bei Bakterienextrakten, Stoffwechselprodukten und Toxinen. Bei den durch filtrierbare Vira hervorgerufenen Krankheiten wird die Unsicherheit der Dosierung durch das Mitverimpfen unverhältnismässig grosser Mengen von Ballaststoffen mit dem immunisierenden Virus noch erhöht.

Ausgegangen wird von dem Grundsatze, dass eine Immunisierung, auch nicht die beste und wirksamste, jemals einen 100 prozentigen Erfolg zu gewährleisten vermag.

Wohl kaum eine andere tierische Infektionskrankheit ist sonst noch durch Schutzimpfungen so günstig zu beeinflussen, wie der Schweinerotlauf. Das Schweinerotlauf-Heilserum ist eine der wenigen Ausnahmen, in denen ein antibakterielles Serum im lebenden Organismus binnen kürzester Zeit eine vollkommene Unschädlichmachung der spezifischen Krankheitserreger zu bewirken vermag.

Aehnlich günstig liegen die Verhältnisse bloss nur noch beim Milzbrand. An die Stelle der Pasteurschen Methode is allmählich die minder gefährliche, aber auch weniger kräftig immunisierende Simultanimpfung nach Sobernheim getreten. Als bedeutender Fortschritt hat die Schutzimpfung mit Saponin-Milzbrankulturen nach Mazzucchi zu gelten. Der Grad der auf diese Impfung hin eintretenden Immunität ist ein hoher (Hruška, Gerlach). Nur Pferde zeigen sich oft überempfindlich gegen Saponin-Kulturen.

Bei den Heilimpfungen mit Milzbrand-Hochimmunserum wird darauf hingewiesen, dass in solchen Fällen, in denen die Heilwirkung des Serums zu versagen scheint, ein voller Erfolg noch erzielt werden kann, wenn den kranken Tieren ausser dem Immun-Serum auch noch frisches Komplement in Form von frischem Rinderblut oder Serum injiziert wird.

Die Erfolge der Rauschbrandschutzimpfungen werden günstig beurteilt, jedoch wird empfohlen, die empirische Methode der Muskelpulverimpfung zu verlassen und an ihrer Stelle die Schutzimpfungen mit Kulturfiltraten nach Gräub und Zschokke und Naoshi *Nitta* bezw. jene mit Formolkulturen in Anwendung zu nehmen. Die Heilwirkung von Rauschbrandimmunserum ist eine ansehnliche, kommt aber bei dem rapiden Kranheitsverlauf meist zu spät.

Es wird ferner auf die Vorteile einer Prophylaxe mit Tetanusantitoxin hingewiesen.

Was die Schweinepestimpfungen anlangt, so sind der Anwendung von Immunserum allein enge Grenzen gesetzt. Die Erfolge der Simultanimpfung, die zu einem lange dauernden Impfschutz führt, sind ausgezeichnet. Die Vakzineimpfungen gegen Schweinepest scheinen, trotzdem diese Ansicht viele Gegner findet, in solchen

Schweinebeständen erfolgreich anzuwenden zu sein, in denen ein kurz (3-5 Monate) dauernder Impfschutz ausreicht, ferner in solchen mit chronischer Ferkelpest und bei Schutzimpfungen von Mastbeständen. Der Impfschutz ist ein relativer und wird von massiven Infektionen leicht durchbrochen.

Die Bekämpfung der Maul- und Klauenseuche mit Hochimmunserum liefert anerkannt gute Erfolge. Doch haften dieser Impfung alle Nachteile einer passiven Immunisierung an. Anzustreben wäre eine aktive Immunisierungsmethode.

Die Impfmethoden von Curasson und von Süreyya Tahssin mit Organvakzinen gegen die Rinderpest bewähren sich vorzüglich.

Die B.C.G.-Impfungen gegen die Tuberkulose der Rinder erfahren noch keine einheitliche Beurteilung, werden aber nach den eigenen, auf mehr als 10 Jahre zurückreichenden Erfahrungen empfohlen.

Gegen eine Reihe von Jungtierkrankheiten verfügen wir über gut wirksame Impfstoffe für die Impfung der trächtigen Muttertiere sowohl als auch der Neugeborenen.

Zu einem völligen Misserfolg haben alle bisherigen Impfmethoden geführt, die sich die Bekämpfung des Bangschen Abortus zur Aufgabe gemacht haben, Hier werden neue Wege zu beschreiten sein.

Sehr wechselnde Erfolge liefern die bisher gebräuchlichen Schutzimpfungen gegen die Geflügelcholera. Ausserdem spielen die hohen Kosten dieser Impfungen eine sehr erhebliche Rolle.

Ueber den Wert oder Unwert der Schutzimpfungen gegen die Tollwut gehen die Meinungen vorläufig noch auseinander.

Die Schutzimpfungen gegen die Lungenseuche haben sich nicht nur unwirksam, sondern geradezu gefährlich erwiesen.

Bei einer Beurteilung der Brauchbarkeit von Schutzimpfungen gegen Pocken, sowie gegen Geflügeldiphtherie und Geflügelpocken empfiehlt sich einstweilen noch Zurückhaltung.

Abschliessend wird das grosse Arbeitsgebiet umrissen, auf dem die Mikrobiologen in Zukunft noch viel Betätigung finden, durch *Vervollkommnung bereits bestehender und Auffindung neuer* Impfmethoden.

Dr. Charles Hruška (Ivanovice, Czechoslovakia): *Sur l'immunité active et passive et sur le rôle du traumatisme dans le charbon.*

(1) L'immunité active dans le charbon est un cas particulier de digestion locale—de là l'immunité locale et générale. Une bonne immunité active dépend de la virulence fixée et de la quantité du virus-vaccin, injecté par n'importe quelle voie (la voie souscutanée ou cutanée).

(2) La production du bon sérum anticharbonneux est étroitement liée non seulement aux bactéridies virulentes mais aussi aux agressines-plasmoréagines. Ce sérum dénommé antiagressique et antibactérien manifeste une supériorité très marquée par rapport aux

meilleurs sérums utilisés par le procédé de Marchoux. Avec le titrage " en gouttes " de culture virulente en bouillon chez le cobaye on peut obtenir des résultats quantitatifs.

(3) Le charbon est une infection qui repose dans la plupart des cas sur le traumatisme de n'importe quel tissu. Le rôle du traumatisme est d'une importance extraordinaire et cette constatation nous permet de lutter avec succès contre l'infection charbonneuse.

Prof. St. Angeloff (Sofia): *Resultate der Serumbehandlung von Milzbrandinfektionen beim Menschen und Milzbrandschutzimpfungen bei Tieren in Bulgarien.*

Der Milzbrand ist in Bulgarien ziemlich stark verbreitet, z.B. sind 1935 2,534 Tiere an Milzbrand zu Grunde gegangen. Bei dieser starken Verbreitung des Milzbrandes unter den Tieren kommt die Infektion auch beim Menschen ziemlich häufig vor. In den letzten zehn Jahren sind 7,241 Menschen an Milzbrand erkrankt und 8.2 prozent von diesen gestorben. (Behandelte sind inbegriffen.) Seit einigen Jahren hat die Serumbehandlung die anderen Behandlungsmethoden fast gänzlich verdrängt; meistens wird das von dem zentral-veterinär-bakteriologischen Institut, Sofia, hergestellte Serum verwendet. Die Resultate der letzten 5 Jahre zeigen, dass von 2,981 Kranken 2,820 geheilt worden sind, also 94.6 prozent. Die Letalität beträgt 5.4 prozent. Nach Sclavo beträgt die Letalität 6.9 prozent bei den Serumbehandelten gegen 24.16 prozent bei den Nichtbehandelten. Nach diesen Angaben kann man die Resultate als gut bezeichnen. Es muss dabei betont werden, dass in den Krankenhäusern oft sehr schwer Erkrankte in moribundem Zustand eingebracht werden, bei welchen kein Erfolg von der Serumbehandlung zu erwarten ist. Diese erhöhen selbstverständlich die Sterblichkeitsprozente.

Wir wollen auch darauf hinweisen, dass es vorteilhaft ist, bei der Serumbehandlung grössere Serumdosen anzuwenden, besonders bei der ersten Injektion (50-100 ccm. intravenös). Die intravenöse Anwendung des Serums ist vorzuziehen.

Von 1931 bis Ende 1935 sind in Bulgarien 500,127 Tiere gegen Milzbrand vacciniert und 21,673 serovacciniert worden; die Mortalität betrug 0.05 bzw. 0.19 prozent.

Drs. G. Ramon and **A. Staub** (Garches) [Communicated by Prof. A. Boivin (Garches)]: *Essais sur l'immunisation contre le charbon sur une nouvelle formule de vaccination charbonneuse.*

La méthode de vaccination charbonneuse de Pasteur, Chamberland et Roux, géniale dans sa conception, n'est pas restée à l'abri de toute critique au cours de son application s'étendant à l'heure actuelle sur un demi-siècle.

Les procédés proposés depuis—tel celui de " l'inoculation intracutanée " ou celui des " vaccins glucosidés "—qui ne comportent qu'une seule injection et qui utilisent soit le deuxième vaccin de Pasteur, soit la bactéridie virulente elle-même, ne sont pas toujours inoffensifs et leur efficacité est parfois relative.

L'emploi d'un virus-vaccin comparable, dans son degré d'atténuation, au " premier vaccin pastorien " offre des garanties réelles d'innocuité. Un tel vaccin présente, en effet, une grande " marge de sécurité " entre son degré d'atténuation et le niveau de son pouvoir pathogène éventuel.

Il est démontré que ce vaccin, enrobé par exemple dans la lanoline, confère, en injection sous-cutanée, une immunité qui permet aux moutons ainsi vaccinés de résister à l'infection expérimentale, mortelle pour les moutons témoins non vaccinés. Cette immunité est dans son installation, dans son mode d'action assimilable, par certains côtés, à l'immunité antidiphtérique obtenue de façon analogue au moyen de bacilles vivants injectés à l'animal d'expériences après incorporation dans les corps gras. Elle est sous la dépendance d'un anticorps qui permet le libre jeu des défenses naturelles (phagocytose) de l'organisme.

Le même vaccin, non plus lanoliné, mais " gélosé et aluné " se montre également très efficace. La formule de l'injection sous-cutanée unique de vaccin gélosé et aluné, est commode à réaliser, elle est inoffensive, elle procure aux animaux une immunité très précoce (effective dès le 5e jour) solide (vis-à-vis des infections virulentes sévères), durable (au delà de six mois). Elle mérite donc d'être adoptée dans la pratique courante.

Dr. Ch. Hruška (Ivanovice, Czechoslovakia): *Essais sur l'immunisation contre le charbon sur une nouvelle formule de vaccination charbonneuse.*

Ramon et Staub tendent à montrer que le premier vaccin anticharbonneux de Pasteur enrobé soit dans la lanoline soit dans la gélose et l'alun sera inoffensive et efficace pour la pratique. D'après notre avis la modification de la première vaccination de Pasteur préconisée par les auteurs va nous apporter d'une part des réactions graves sur des animaux vaccinés (la dissociation de premier vaccin charbonneux de Pasteur), et d'autre part que le premier vaccin originel de Pasteur même enrobé dans les différentes substances n'est pas capable de nous donner une immunité satisfaisante et durable dans la pratique. D'après les résultats indiqués il n'y a pas de différence entre la vieille formule des savants qui ont trouvé de bons résultats sur les moutons avec les vaccins dits " uniques " et la nouvelle formule de vaccination charbonneuse, préconisée par Ramon et Staub.

Dr. L. B. Bull (Melbourne) [Communicated by Dr. G. F. Petrie (Elstree, England)]: *Infection by and Resistance to the Preisz-Nocard Bacillus* (Corynebacterium ovis) *in Experimental Animals.*

The Preisz-Nocard bacillus, the cause of caseous lymphadenitis of sheep, is capable of forming a relatively strong toxin in culture media. The guinea-pig is highly susceptible to infection by this bacillus. By the use of formol-toxoid a high degree of antitoxic immunity can be produced in the guinea-pig. Such animals, although resistant to 30 M.L.D. or more of toxin, can be infected with minimal doses of culture or pus suspensions with the same degree of certainty as can normal guinea-pigs, and the disease produced will progress with the formation of distant metastases. These immune animals are not susceptible to early death from doses of culture which will kill normal guinea-pigs in twenty-four to seventy-two hours. It is clear that delayed death is not a measure of resistance to initial infection.

No method of vaccination by mixtures of formol-toxoid and formol-killed bacilli, or by heat-killed bacilli alone, has given any definite degree of immunity in guinea-pigs against infection by the Preisz-Nocard bacillus. Anti-sera, produced by intravenous injection of calves or sheep with heat-killed or live bacilli over long periods, give no protection against infection in the guinea-pig whether the sera be given by subcutaneous injection or be mixed with the infecting dose beforehand, in spite of the fact that agglutinins, precipitins, and, when the antigen is the live bacillus, antitoxin, are present in the sera.

Immunity is a complex phenomenon. With many micro-organisms resistance to the more or less immediate lethal effects of infection enables the animal body to destroy the infecting micro-organisms. This resistance may reside mainly in the presence of an antitoxic immunity, but where, as in the case of the Preisz-Nocard bacillus, the establishment of infection and not the immediate morbidity is the criterion of immunity, antitoxins may play little or no part.

The present investigations suggest that antitoxic immunity may not protect against infection but may greatly reduce the severity of the morbid processes. For practical purposes in special cases, nothing more may be necessary in prophylaxis.

Dr. N. S. Ferry (Detroit, U.S.A.): *Meningococcus Antitoxin: Laboratory and Therapeutic Status.*

Experimental Development.—The rationale for the use of meningococcus antitoxin in the treatment of meningococcal meningitis is based on the following findings:

(1) Broth filtrates of young cultures of the meningococcus contain exotoxins specific to the individual types.

(2) Toxin is neutralized by homologous convalescent sera.

(3) Toxin produces typical symptoms of meningitis when injected intracisternally into certain laboratory animals.
(4) Toxin stimulates antitoxins specific to the several types when injected subcutaneously.
(5) The human, the natural host of the meningococcus, is extremely susceptible to the toxin, as determined by intradermal injections of diluted toxin.
(6) Active immunity against this dose of toxin has been produced in susceptible individuals by means of subcutaneous injections of increasing amounts of undiluted toxin.
(7) Monkeys have been made immune to intraspinal inoculations of fatal doses of live meningococci, following recovery from intracisternal injections of toxin in doses sufficient to produce severe symptoms typical of meningitis.
(8) Meningococcus antitoxin has protected guinea-pigs, prophylactically and therapeutically, against the fatal intraperitoneal dose of live meningococci.
(9) Antitoxin has saved a large percentage of monkeys against intraspinal inoculations of the fatal dose of live cultures, when injected intraperitoneally after critical symptoms have appeared.
(10) Experiments on antitoxin were controlled with antimeningococcic sera, with negative results.

Clinical Application.—Reports from a large number of cases treated with meningococcus antitoxin during the past two years, in several sections of the United States of America, have shown that the mortality rate has been decreased, on an average, 50 per cent., and that hospitalization has been markedly shortened.

Injections of antitoxin have been given (*a*) intraspinally, combined with large doses intravenously, (*b*) intravenously alone, with lumbar puncture for diagnosis and drainage, and (*c*) intravenously alone without lumbar puncture, diagnosis being made by blood culture.

Dr. B. G. Maegraith (Oxford): *Immunity to Meningococcus.*

It is possible to produce poisonous filtrates of meningococci of both main groups. These filtrates will kill guinea-pigs if injected subdurally or intracisternally in as small doses as 0.2 to 0.02 cc. Even as much as 10 cc. of the filtrate injected intraperitoneally into a guinea-pig will often fail to kill. Intravenous doses are similarly relatively inactive. No serum I have tried has given the slightest indication of neutralizing the poisonous substances in the filtrates, at least, when injected subdurally with the filtrates. I have tried "antitoxic" serum, prepared by Dr. Petrie, by injection of a horse with filtrates from English strains and three specimens of "antitoxic" sera from Dr. Ferry, in addition to several "antibacterial" therapeutic sera, with the same results.

The poisonous substances in the filtrates produce tremendous vasodilation, particularly in the substance of the brain and in the lungs. In this respect the effects of the poisonous filtrates are essentially similar to those produced by subdural injection of extracts of meningococcal cells. Toxic filtrates which produce identical lesions can be obtained from 3 to 5 day blood broth cultures of *N. gonorrhœæ, N. catarrhalis* and *N. pharyngis*. Subdural injection of trypan blue produces similar changes in the brain.

I think, therefore, that the meningococcal filtrates are non-specific in their effects and non-antigenic as far as their toxic properties are concerned. If a " true toxin " was present in the filtrate I believe " antitoxic " sera would neutralize it and protect laboratory animals from the effects of the injection.

Dr. G. F. Petrie (Elstree, England) : *The Endotoxin of the Meningococcus.*

When the guinea-pig, the rabbit and the horse were treated with repeated doses of endotoxin preparations in the form of suspensions or extracts, tests for a resultant immunity failed to give any evidence of tolerance to the pathogenic action of the toxin. Moreover, the serum obtained from the treated animals did not confer immunity against the endotoxin when serum-toxin mixtures were injected intracerebrally or intracutaneously into the guinea-pig. In contrast to these results, comparable experiments with three neurotoxins, namely, diphtheria toxin, tetanus toxin and scorpion venom, showed that there was *no* difficulty in neutralizing the pathogenic action of from 20 to 100 intracerebral lethal doses of these neurotoxins when specific serum-toxin mixtures were injected directly into the brain of the guinea-pig. Thus the conclusion may be drawn that the meningococcal endotoxin is not antigenic. The importance of the endotoxin as a pathogenic agent is not lessened by this circumstance, since the lesions of cerebro-spinal fever are probably attributable to it. The efficacy of the specific treatment in man is apparently due in large measure to an antibacterial mechanism.

Prof. M. Ciuca and **Prof. I. Nicolau** (Bucarest) : *Efficacité de la sérothérapie dans la méningite cérébro-spinale épidémique.*

Au cours de 15 années, des résultats meilleurs en Roumanie que dans d'autres pays.

Collaboration entre l'Institut Cantacuzène et trois hôpitaux (Clinique infantile du Prof. Manicatide ; l'Hôpital Izolarea, Prof. Balteanu ; l'Hôpital militaire " Regina Elisabeta," Médecin Colonel Popesco). On a fixé les principes de la préparation du sérum et la méthode de son application dans le traitement. Trois mémoires concernant les détails étaient à la disposition des membres de la 8-ème section.

Préparation du sérum (mono- et polyvalent). Souches typiques de l'Institut Pasteur et l'Institut Lister, et souches indigènes constamment renouvelées. La vaccination des chevaux, avec des germes vivants d'après la méthode d'Amoss et Wollstein, dure en général 90 jours. La saignée est pratiquée 8 et 12 jours après la dernière injection. Le sérum est chauffé une heure à 56° C., 2 jours de suite. Pas d'antiseptiques. Durée d'efficacité du sérum 5 mois (après la saignée).

Méthode de traitement. La sérothérapie intrarachidienne ou intraventriculaire (nourrissons) journalière, *précédée d'un examen* cyto-bactériologique du liquide céphalo-rachidien ; ceci nous permet d'inoculer des quantités—juste suffisantes—pour guérir le malade et éviter la redoutable méningite sérique.

Aussi, la sérothérapie intramusculaire ou endoveineuse (méningococcémie) et au besoin l'autovaccinothérapie.

Résultats thérapeutiques.[1]—*Total des cas :* 230 (71.3 pour cent mâles). 0-1 an : 67 ; 1-5 ans : 52 ; 5-10 ans : 16 ; 10-20 ans : 20 ; plus de 20 ans : 75.

127 cas : traitement dans les premiers 6 jours ; pour le reste après le 6ᵉ.

186 ont guéri (80.8 pour cent). La guérison était effective en 50.9 pour cent après 4 injections intrarachidiennes.

Les quantités totales de sérum injectées dans le canal rachidien variaient de 50-400 cm.c. par malade. Dans 83 pour cent la quantité moyenne de sérum ne dépassait pas 200 cm.c.

Dr. E. L. Sturdee (London): *The Serum Treatment of Cerebro-spinal Meningitis.*

The difficulty of assessing the real value of a therapeutic agent is well known, and the figures I propose to give are subject to that reservation. A request was made by the Ministry of Health for records of proved cases of cerebro-spinal fever treated intrathecally with anti-meningococcus serum ; 2,901 such records have been received. After excluding deaths due to intercurrent disease or occurring within 24 hours of the first dose of serum, 2,625 cases remained, of which 971 (36.99 per cent.) died. Flexner (1913) stated that the fatality rate before the introduction of serum treatment was 70 to 80 per cent., and Dunn (1908) found 58 to 80 per cent. In a series of 382 cases, which received no serum intrathecally, I myself found a fatality rate of 61.87 per cent.

Early and late treatment : Among the 2,625 cases under consideration, 1,788 treated during the first week had a fatality rate of 30.9

[1] Répandus dans tout le pays. Cas sporadiques. La virulence de la maladie ne paraît pas être inférieure à celle d'autres pays ; cependant le pourcentage de cas de méningococcémie ne dépasse pas 5 pour cent de nos cas. La léthalité pour tout le pays (cas traités ou non) a été dans les dernières années de 56.2 pour cent dans la campagne et de 45.4 pour cent dans les villes.

per cent. and 837 treated on, or after, the seventh day had a fatality rate of 50.1 per cent.

Circular of 1932: Because of the variation in dosage of serum and intervals between injections in different hospitals, the Ministry of Health issued a circular drawing attention to the suggestions for the use of serum given by Flexner (1908). A comparison of his records and my records collected from hospitals in which his suggestions were normally followed, shows considerable agreement as may be seen from the following figures:

Flexner's records—1,294 cases, 400 deaths (30.9 per cent.)
My records —1,081 cases, 298 deaths (27.6 per cent.)

These figures tend to show that serum treatment is at least as useful now as in 1913.

Age periods: The cases fall into three groups with the fatality rates as follows:—

Under 5 years—903 cases with a fatality rate of 46.6 per cent.
Over 20 years—687 cases with a fatality rate of 42.8 per cent.
5 and under 20 years—1,028 cases with a fatality rate of 24.6 per cent.

It seems reasonable, therefore, that, in analyzing any series of figures, the age periods of the patients should be taken into consideration.

Conclusions: I am afraid it cannot be claimed that this investigation has produced any advance on existing knowledge, but in my opinion it tends to show that provided the patient comes under treatment at a reasonably early moment, is of suitable age, and receives adequate amounts of serum prepared from current strains of meningococci he has a reasonable chance of recovery; also, that intrathecal treatment with anti-meningococcus serum is the most hopeful means of obtaining a good result.

Dr. Sara E. Branham (Washington, D.C.): *Antitoxic and Antibacterial Immunity in the Prophylaxis and Treatment of Meningococcus Meningitis.*

For prophylaxis in meningococcus meningitis passive immunization has not been employed and active immunization has not had adequate trial. Vaccination has been attempted in several groups, but the results have been inconclusive. Recently, skin testing of groups of individuals with the toxins of the meningococcus, and the immunization of those individuals reacting to it by a series of injections of the toxins, have been employed. This has not yet been done extensively enough for its significance to be known.

In the treatment of active cases of meningitis, both antibacterial and antitoxic sera are now being used. Opinions as to the relative value of these two types of sera cannot be given at the present time, since antitoxin therapy may be said to be still in the experimental stage. Satisfactory methods of standardization are lacking for both types of serum, and until such methods are developed the

eal value of serum therapy in meningococcus meningitis will not be certainly known.

Miller has shown that suspension of meningococci in mucin emphasizes any virulence which a culture may possess, and it now seems possible to compare the protective value of anti-meningococcic serum. Different lots of serum vary widely in protective action, though the agglutinin titre of all may be high. Whether the results in mice will be comparable to those obtained in treating patients can only be determined later.

Various lots of Ferry's antitoxin also vary in efficacy when tested in mice against living culture, and some protect well. Toxin-antitoxin neutralization has not been demonstrated successfully in laboratory animals, the only method of standardizing the antitoxin being by the neutralization of toxin in skin tests in human beings.

Whatever the nature of the meningococcus toxins may be, the toxicity of meningococci cannot be ignored in preparing serum for the treatment of meningococcus infections. Clinical experience, as well as animal experiments, indicate that serum which is both antibacterial and antitoxic is essential.

What rôle does serological type play in serum therapy of meningococcus meningitis ? The general practice is to administer polyvalent serum, but quick typing by means of a precipitation test on the spinal fluid (Rake, 1933) may result in increased use of monovalent serum. This bedside typing can only be applied to a limited number of cases since there must be abundant specific soluble substance in the spinal fluid and the patient must not have had any previous serum therapy.

Dr. C. Phillip Miller (Chicago, U.S.A.) : *Experimental Meningococcal Infection.*

Experimental meningococcal infection has been produced quite regularly in the mouse by intraperitoneal inoculation of meningococci suspended in mucin.

The meningococci are grown a short time (less than 6 hours) on solid media and washed off in saline. This suspension is made up to approximately 1,000 million organisms per cc. and then titrated through a series of ten decimal dilutions with mucin. Mice are then inoculated intraperitoneally with 1.0 cc. of the desired dilutions. Rapid bacteræmia develops and the mice die within 18 to 48 hours, without any evidence of localized meningeal involvement.

The minimal lethal dose of virulent strains is less than 10 organisms although several hundreds of millions are required to infect mice if the meningococci are suspended in saline alone.

The action of mucin is not clearly understood, but seems to involve interference with the defence mechanism of the host rather than enhancement of the virulence of the micro-organisms themselves.

The method has been found useful in the diagnosis of early cases of meningitis when the spinal fluid contains too few micro-organisms to be seen in the centrifuged sediment. We inject such sediments, mixed with mucin, into mice and examine their peritoneal exudates at the end of 5 or 6 hours. In this way meningococci have been identified in the peritoneal exudate of mice some hours before cultures of the sediment have grown or even when they have remained sterile.

Mice can be protected against infection by preliminary injection of immune serum, and they can be spared a fatal outcome if serum is administered in sufficient quantity, even though the infection is quite far advanced. In our experiments, larger quantities of sera prepared by immunization with nucleoprotein were required for protection than ordinary antibacterial sera; still larger quantities of sera prepared by immunization with filtrates of liquid cultures were necessary. All the sera were monovalent and were prepared in rabbits.

Protocols were shown of (*a*) a titration of the protective action of an ordinary antibacterial serum, and (*b*) an experiment to determine the "therapeutic" effect of immune serum on experimental infection.

The mouse protection test has been applied to a study of the antibody content of the sera of patients undergoing treatment for epidemic meningitis. Samples of the patient's serum obtained during the course of the illness were tested against the corresponding strain. The mouse-protective titre rises sharply immediately after the administration of therapeutic serum and declines slowly during convalescence.

Dr. H. J. Parish (Beckenham, England): *Difficulties in Titrating Anti-meningococcal Serum.*

Miller and Branham obtained protection in mice with anti-meningococcal sera against infection with meningococci suspended in mucin. Parish and Proom found that agglutination titres were not a measure of protective activity; concentrated antisera might still protect mice even when agglutinins were absent. One sample of Ferry's "antitoxic" serum they had tried was certainly not superior to antibacterial serum. Miller had found no protection with normal horse serum, whereas Branham's published protocols showed that protective substances were present in a number of batches. In some tests normal horse sera protected as effectively as various anti-meningococcal sera. The speaker's own results with six consecutive batches were similar to Branham's. He would have expected a much greater difference in protective capacity between the sera of normal horses and of horses immunized for long periods with living and heat-killed cultures of meningococci. A satisfactory method of titrating anti-meningococcal sera had not yet been discovered.

Prof. J. W. McLeod (Leeds): *The Part played by Antitoxin in Immunity to different Types of Diphtheria Bacillus.*

In the last four years, observations on the existence of three well-defined cultural types of the diphtheria bacillus have led to the surprising conclusion that there is a group of strains—the "gravis" type—which have been responsible for the most severe epidemic manifestations of diphtheria during that period, but are very poor toxin formers *in vitro*.

These observations have raised the following questions:

(1) Are these bacilli endowed with special invasive properties apart from their capacity to produce toxin?

(2) Do they produce toxic elements other than and distinct from diphtheria toxin as usually understood, and as used to prepare commercial antitoxin?

(3) Do they produce the usual diphtheria toxin better in the body than do the other strains, i.e., are the results obtained *in vitro* reversed *in vivo*?

Observations on protection of laboratory animals from injection of such strains by standard antitoxin have appeared to answer these questions in the sense that these strains depend for their pathogenic effect on the usual diphtheria toxin.

Observations on the protection of the community by toxoid preparations made from such toxin do not, however, support this conclusion, as is shown by the figures given in the table below for the ratio of cases in immunized to other cases in a series of about 4,000.

Type	Number of cases.	Number in immunized or Schick-negative patients.			
		Total.	Mild.	Severe.	Fatal.
"Gravis" ...	2,103	107	60	44	3
"Intermediate"	815	20	12	8	–
"Mitis" ...	961	13	12	1	–

It follows that the "gravis" strain is distinctly more capable of surmounting the barrier of toxic immunity than the other types a conclusion borne out by the work of Dudley, May and O'Flynn (1934) on the incidence of mild diphtheria in a completely immunized community.

It remains to be determined whether this difference is due to special qualities of the bacterial toxin or to other bacterial characters.

Dr. A. Felix (London): *The Serum Treatment of Typhoid Fever.*

It is particularly difficult to assess the relative value of antitoxi and antibacterial mechanisms in immunity to infection with bacteri

which do not produce the so-called typical exotoxin. Antibodies to exotoxins do not exert any direct antibacterial effect, whereas antibodies to the so-called endotoxins are concerned with both antitoxic and antibacterial activities.

B. typhosum is devoid of typical exotoxin, and the two antigenic constituents known as the O and Vi antigens are at present recognized as the essential elements in the pathogenicity and immunogenic value of this organism. The Vi antibody alone confers protection against experimental infection of mice with highly virulent strains which are rich in Vi antigen. The O antibody appears to be chiefly responsible for effecting the neutralization of the endotoxin, although the O antibody also exerts a powerful and direct antibacterial effect on strains which contain less Vi antigen and consequently are less virulent.

The efficacy of a therapeutic antityphoid serum depends on the presence in it of effective amounts of each of the two antibodies. Clinical trials conducted with pure anti-O serum and with serum containing both O and Vi antibodies clearly indicated that the latter produced the more beneficial effects.

It is known that no specific serum treatment of typhoid fever of recognized value has yet been evolved, in spite of many attempts made during the past thirty-five years. All the commercial antityphoid sera which have been tested so far were found to be entirely devoid of the Vi antibody and very poor in O antibody; in the mouse-protection test these sera were of no protective value whatsoever. The Vi antigen is in some respects extremely labile, and this is the reason why antityphoid sera produced according to the customary methods of immunization with killed bacteria or with extracts do not contain any Vi antibody. Alcohol-treated suspensions of Vi-containing bacteria seem to be adequate substitutes for the " natural " Vi antigen contained in the living organisms.

It is suggested that antigenic analysis of other pathogenic bacteria may disclose the presence in them of yet unknown, but essential, antigenic constituents, and may lead to the solution of some of the practical problems of active and passive immunization.

Dr. F. Kauffmann (Copenhagen): *Ueber die Bedeutung des Vi-Antigens für die Typhus-Infektion und Immunität der Maus.*

Damit der Typhusbacillus in der Maus eine krankmachende oder tötende Wirkung entfaltet, muss er in grossen Dosen parenteral injiziert werden, d.h. in Dosen, die eine *akute Intoxikation* erzeugen. Wir können also im Mäuseversuch nur *Toxizitätsunterschiede* zwischen verschiedenen Kulturen feststellen, aber keine Virulenzunterschiede.

Diejenigen Bacillen, die das Vi-Antigen maximal besitzen (V. Formen) unterscheiden sich im Mäusekörper hinsichtlich Vermehrung, Eindringungsfähigkeit, u.s.w., *in keiner Weise* von denjenigen

Bacillen, die das Vi-Antigen nicht enthalten (W-Formen), sofern nicht akut toxische Dosen injiziert werden. Dagegen sind die V-Formen erheblich toxischer als die W-Formen, sodass dem Vi-Antigen eine spezielle Toxinwirkung zugesprochen werden muss.

Bei der *aktiven und passiven Immunisierung* von Mäusen können wir gegen tödliche Intoxikationen mit V-Formen nur durch Vorbehandlung mit V-Formen schützen, nicht dagegen durch W-Formen, denen das Vi-Antigen fehlt. Eine Ausnahme besteht jedoch darin, dass wir auf Grund der Versuche von Ørskov und Kauffmann mit W-Formen ebenfalls aktiv gegen V-Formen immunisieren können, sofern wir die Prüfungsinfektion in den ersten Tagen nach der Schutzimpfung vornehmen. In diesem Falle, in dem es sich um eine Erscheinung nach Art der *Morgenrothschen Depressionsimmunität* handelt, spielt also das Vi-Antigen keine Rolle.

Wir betrachten das Vi-Antigen als normales Körperantigen der Typhusbacillen, das bei der Herstellung von Impfstoffen und Heilseren für menschliche Zwecke berücksichtigt werden muss, auch wenn wir aus den Mäuseversuchen nicht direkt auf den Menschen schliessen und die Bedeutung des Vi-Antigens bei der Typhus-Infektion und Immunität des Menschen nicht kennen. Wir müssen es dagegen ablehnen, das Vi-Antigen als " *Virulenzantigen* " zu bezeichnen. Da aber überhaupt noch keine Salmonella-Antigene nachgewiesen sind, deren Anwesenheit die Virulenz bedingt, so müssen wir den Ausdruck " *Virulenzantigen* " ganz allgemein fallen lassen. Das von Felix und Pitt beschriebene " Vi-Antigen " der Mäusetyphus-Bacillen ist z.B. weder ein Vi-Antigen im Sinne des Typhus Vi-Antigens noch besitzt es eine Bedeutung für die Virulenz.

Durch diese Feststellungen wird die *serologische* Bedeutung der verschiedenen Körperantigene, deren weitere Erforschung unser Ziel sein muss, nicht berührt.

Dr. H. Schütze (London) : *The Sterilization of Antityphoid Vaccine.*

Typhoid Vi antigen has been shown by Felix to be a very important factor in immunity as demonstrated in the mouse. Vi antigen has been shown, also, to be relatively labile, its efficiency as an antibody-producing substance being lowered or altered in varying degree according to the method employed for sterilization of the vaccine. It has become of practical importance to discover in which way a vaccine may be rendered sterile with least damage to the immunizing properties of this Vi antigen. For this purpose batches of mice, varying in number from 50 to 130, were immunized with vaccines prepared from a Vi-containing strain of *S. typhi* and sterilized in a variety of ways. These mice were subsequently injected with a test dose of the same Vi strain, representing three to four M.L.D. The percentage of survivors furnished evidence of the prophylactic efficacy of the vaccines employed. The methods of sterilization compared were: (1) heating to 58° for 1½ hours;

(2) heating to 53° with subsequent addition of 0.5 per cent. phenol; (3) 0.5 per cent. phenol at room temperature; (4) 1 in 4,000 merthiolate at 37°; (5) 1 in 500 formalin at 37°; (6) 96 per cent. alcohol at room temperature; (7) CO_2, 60 atmospheres pressure at room temperature; (8) 75 per cent. saccharose at 37°; (9) 25 per cent. galactose at 37°; (10) 20 per cent. NaCl at 37°. No vaccine was seen to give definitely better protection than any other. In other words, all these methods of sterilization produced vaccines of equal anti-Vi prophylactic power. The significance of such results depends on the precision with which antigenic differences can be detected. A comparison of the immunity occasioned in 90 mice receiving 50 per cent. more of a certain vaccine than did 90 others showed that this quantitative difference has reached the limits of detectability, for 24 per cent. more survivals occurred among the mice receiving the 50 per cent. greater amount of vaccine.

Dr. Al. Slatineanu, Prof. I. Balteanu and **Dr. I. Alexa** (Iasi, Roumania) [Communicated by Prof. M. Ciuca (Bucarest)]: *Typhoid Fever in Vaccinated Persons.*

Many authorities report frequent typhoid infections among vaccinated persons.

We have analyzed observations concerning 698 typhoid patients in the Contagious Diseases Hospital in Iasi, all diagnosed by blood culture. Thirty-eight had had a full course of inoculations, viz., 24 patients had been vaccinated more than four years previous to their illness, and 14 within the previous four years. In the latter group two patients had been vaccinated one year before the onset of the disease.

In the first group there were 13 mild cases, 9 severe cases, 2 fatal cases. In the second group there were 7 mild cases, 5 severe cases, 2 fatal cases (one vaccinated one year prior to the onset of the disease).

The frequency of typhoid fever among persons who had received the full course of inoculations and the occasional severity of the disease show that the method is not perfect, although it does reduce morbidity. It must be emphasized that the virulence of strains employed in the preparation of vaccines constitutes an important factor. We believe it necessary to use vaccines prepared from local strains frequently replaced by freshly isolated cultures. Strains recently isolated presumably have an average greater virulence.

We recommend that the number of inoculations be increased from 3 (0.5 cc. [500,000,000], 1 cc. and 1.5 cc.) to four or even to five inoculations. With regard to annual revaccination, it is sufficient to give a single injection of 1 cc., and not the usual full course.

These observations were carried out at the Contagious Diseases Hospital, Iasi, Roumania.

*Dr. **H. Ghosh** (Calcutta) : *Treatment of Cholera with Anticholera Serum.*

An antitoxic cholera serum was prepared by immunizing horses with a toxin obtained by anaerobic culture in highly alkaline broth (pH 8.4 to 8.8)[1]. It was given trial during two epidemics in Calcutta in 1934 and 1935. The mortality among serum-treated cases was 10.65 per cent. and in control cases 21.6 per cent.

The serum was injected in large quantity (about 75 to 80 cc.) *intraperitoneally*, so as to bring the specific serum rapidly into contact with the lesion and to have it absorbed through the portal system. Since the course of cholera is extremely rapid, this was considered a rational procedure.

Results were undoubtedly specific in cases treated within 3 or 4 hours of onset.

In a series of 258 patients treated with 25 cc. to 80 cc. of serum intraperitoneally, anaphylaxis was not encountered ; only two cases showed evidence of serum sickness in the form of mild urticaria. The time taken for the serum to be absorbed was 4 to 6 hours according to the quantity. Apparently large quantities of whole immune serum can be injected intraperitoneally without much risk of anaphylaxis or serum sickness.

Dr. F. Gerlach (Mödling bei Wien) : *Über Erfolge Unzulänglichkeiten und Misserfolge der wichtigsten Schutz- u. Heilimpfungen gegen tierische Infektionskrankheiten.*

Die Ausführungen behandeln die bereits im vorläufigen Programm aufgezählten Punkte in ausführlicher Weise, mit der Absicht, weniger zu loben, als vielmehr zu bemängeln und anzuspornen zur Vervollkommnung derzeit in der Tierheilkunde bestehender u. geübter Impfmethoden. Es bedarf noch mannigfaltiger Verbesserungen, selbst bei längst wohl erprobten Impfverfahren. Unerfreulich ist die Tatsache, dass wir bei einer nicht geringen Zahl von tierischen Infektionskrankheiten über Immunisierungsverfahren derzeit überhaupt noch nicht verfügen (infektiöse Anämie, Psittakose u.a.m.).

Dem Mikrobiologen steht demmach für die weitere Zukunft in der Verbesserung alter und in der Schaffung neuer Immunisierungsmethoden ein grosses Arbeitsgebiet offen.

Dr. R. A. O'Brien, the Chairman, also took part in the Discussion.

[1] Ghosh, *C. R. Soc. de Biol.*, 1933, CXII, 1176.

CENTRAL INTERNATIONAL COMMITTEE AND PERMANENT COMMISSION OF THE INTERNATIONAL SOCIETY FOR MICROBIOLOGY

A meeting of the Central International Committee and the Permanent Commission of the International Society for Microbiology took place at University College, London, on Tuesday, July 28, 1936, at 2.30 p.m.

The following members were present: Prof. J. C. G. LEDINGHAM (Great Britain), Chairman; Prof. J. BORDET (Belgium), Prof. M. CIUCA (Roumania), Prof. J. FORSSMAN (Sweden), Prof. A. J. KLUYVER (Holland), Prof. TH. MADSEN (Denmark), Prof. F. MESNIL (France), Dr. T. M. RIVERS (U.S.A.), Prof. O. STRENG (Finland); with Prof. R. OTTO (Germany), Dr. P. FILDES AND Dr. W. M. SCOTT (Great Britain) as observers; and Dr. R. ST. JOHN-BROOKS as acting Secretary.

A full discussion took place on the functioning of the International Society according to its statutes since their formulation at the First Congress in Paris in 1930. (These statutes are printed in Tome II of the Proceedings of the Paris Congress.)

The discussion arose on a memorandum prepared by the Executive Committee of the London Congress and presented by the Chairman, the President. In this memorandum it was pointed out that the retention of the International Society, at any rate in its present form, was undesirable on the ground that it had failed, and probably always would fail, to secure the adherence of individuals of all nations from whose enrolment-inscription funds could be built up and retained by its Permanent Commission for the various purposes contemplated in the Paris Statutes. The Society so far had been able to perform only one—and that its most important—function, viz. the securing of the continuity of congresses.

In these circumstances the memorandum of the British Executive Committee went on to suggest that the Society be dissolved and that, in its place, a Permanent International Commission for the organization of triennial International Congresses be appointed.

It was generally agreed that the suggestion put forward by the British Committee had much force behind it but that a motion to dissolve the Society, if carried, might do injury to such international co-operation in the work of organizing congresses which had been achieved by National Committees in various countries adhering to the Society, though it was freely admitted that many of these adhering National Committees had functioned but little, if at all, as international centres of liaison, either with their own national societies or with the Secretary-General's Office in Paris.

After discussion it was resolved to put forward to the Members f Congress at the Plenary Meeting on Saturday, August 1, 1936 ertain Resolutions for general approval. These Resolutions, which re set out in full on pages 27 and 28 of these Proceedings, were ubsequently passed by the Plenary Congress.

NOMENCLATURE COMMITTEE

Members of the Nomenclature Committee of the International Society for Microbiology at the time of the Second International Congress for Microbiology, London, 1936 :—

AUSTRIA : Prof. A. Janke (Vienna).
BELGIUM : Prof. D. Bruynoghe (Louvain), Prof. E. Renaux (Brussels).
BULGARIA : Prof. W. Markoff (Sofia), Prof. T. Petroff (Sofia).
CANADA : Dr. W. L. Holman (Toronto), Dr. G. B. Reed (Kingston).
DENMARK : Prof. M. Christiansen (Copenhagen), Dr. Vilh. Jensen (Copenhagen), Prof. S. Orla-Jensen (Copenhagen).
FRANCE : Dr. M. Langeron (Paris), Prof. F. Mesnil (Paris), Dr. A. R. Prévot (Vanves).
GERMANY : Prof. K. B. Lehmann (Würzburg), Prof. Reiner Müller (Köln).
GREAT BRITAIN AND IRELAND : Mr. J. Ramsbottom (London), Dr. R. St. John-Brooks, Joint Hon. Secretary (London), Dr. W. M. Scott (London), Dr. A. C. Thaysen (Teddington), Mr. P. Bruce White (London).
HOLLAND : Prof. H. Aldershof (Utrecht), Prof. P. C. Flu (Leiden), Prof. A. J. Kluyver (Delft).
HUNGARY : Prof. R. Manninger (Budapest).
IRISH FREE STATE : Dr. M. Grimes (Cork).
ITALY : Prof. Azzo Azzi (Torino), Prof. G. de Rossi (Perugia).
NORWAY : Dr. A. de Besche (Oslo), Dr. J. Voss (Oslo).
PALESTINE : Dr. M. ASHNER (Jerusalem).
POLAND : Prof. F. Eisenberg (Cracow), Prof. Z. Szymanowski (Warsaw).
ROUMANIA : Prof. C. Ionescu-Mihaiesti (Bucharest), Prof. G. Zotta (Bucharest).
SPAIN : Dr. F. Murillo (Madrid), Dr. A. Ruiz Falcó (Madrid).
SWEDEN : Prof. C. Barthel (Stockholm), Prof. G. Hülphers (Stockholm), Prof. A. Pettersson (Stockholm).
SWITZERLAND : Prof. R. Doerr (Basel), Prof. W. Frei (Zurich).
UNITED STATES OF AMERICA : Prof. R. S. Breed, Joint Hon. Secretary (Geneva), Dr. J. Howard Brown (Baltimore), Prof. R. E. Buchanan (Ames), Dr. I. C. Hall (Denver), Prof. E. A. Pribram (Chicago), Prof. O. Rahn (Ithaca).
U.S.S.R. : Prof. B. Issatchenko (Moscow)

MEETING BEFORE THE OPENING OF THE CONGRESS.

An informal meeting of the Nomenclature Committee, at which members were entertained to afternoon tea by Mr. J. Ramsbottom, took place at the British Museum (Natural History), London, S.W., on Saturday, July 25th at 4.0 p.m.

The following members were present: Prof. R. S. BREED (U.S.A.), Chairman; Prof. J. HOWARD BROWN (U.S.A.), Prof. R. E. BUCHANAN (U.S.A.), Dr. M. GRIMES (Irish Free State), Prof. A. J. KLUYVER (Holland), Prof. S. ORLA-JENSEN (Denmark), Prof. A. PETTERSSON (Sweden), Dr. A. R. PRÉVOT (France), Prof. E. A. PRIBRAM (U.S.A.), Mr. J. RAMSBOTTOM (Great Britain), Dr. W. M. SCOTT (Great Britain), Dr. A. C. THAYSEN (Great Britain), Mr. P. BRUCE WHITE (Great Britain).

The following members of the Congress were also present: Dr. P. ARNE HANSEN (Denmark), Dr. F. KAUFFMANN (Denmark), Dr. H. SCHÜTZE (Great Britain).

Informal discussions took place and the time, place and programme of the next meeting were arranged.

MEETINGS DURING THE CONGRESS

A meeting of the Nomenclature Committee was held in the Congress Committee Room at University College, London, W.C., on Monday, July 27th, at 4.30 p.m. The following members were present:—Prof. R. S. BREED (U.S.A.), Chairman; Prof. R. E. BUCHANAN (U.S.A.), Dr. M. GRIMES (Irish Free State), Prof. A. J. KLUYVER (Holland), Prof. F. MESNIL (France), Prof. S. ORLA-JENSEN (Denmark), Prof. A. PETTERSSON (Sweden), Dr. A. R. PRÉVOT (France), Prof. E. A. PRIBRAM (U.S.A.), Mr. J. RAMSBOTTOM (Great Britain), Dr. R. ST. JOHN-BROOKS (Great Britain), Dr. W. M. SCOTT (Great Britain), Dr. A. C. THAYSEN (Great Britain), Mr. P. BRUCE WHITE (Great Britain).

The following members of the Congress were also present: Dr. J. O. W. BLAND (Great Britain), Dr. P. ARNE HANSEN (Denmark), Dr. F. KAUFFMANN (Denmark), Prof. C. B. VAN NIEL (HOLLAND and U.S.A.), Dr. J. C. N. PENIDO (Brazil), Dr. H. SCHÜTZE (Great Britain).

The statement with regard to the constitution and functions of the Nomenclature Committee drawn up by the Nomenclature Commission and approved at the First International Congress for Microbiology in Paris at its Plenary Meeting on July 25th, 1930 (see 1er *Congrès International de Microbiologie*, Tome II, Section VII, p. 519, *et seq.*) was presented by Professor Buchanan, Dr. Prévot and Prof. Pribram. This was felt by those present adequately to outline the functions of the Nomenclature Committee.

Mr. Bruce White next presented a paper entitled "Remarks on the Nomenclature and Classification of Bacteria" (*see Appendix, page* 547). A communication entitled "Vorschläge und Fragen an

das Salmonella-Subkomitee, die zur Diskussions-Basis dienen sollen" by Dr. Kauffmann was also presented in mimeographed form. Mr. Bruce White then led a discussion on criteria to be used in classification, in which Prof. Kluyver, Dr. Prévot, Dr. Buchanan, Mr. Ramsbottom, Dr. Scott, Dr. Thaysen, and others took part.

The Committee welcomed Dr. Penido (Brazil) as a member of the Nomenclature Committee representing that country.

The meeting then adjourned to examine the film entitled "Germination of Spores of Bacteria" by Prof. S. Bayne-Jones (Yale University, U.S.A.), which was shown by Dr. Bland and explained by Prof. Breed. The film showed very clearly that the spores of the Marburg strain of *Bacillus subtilis*, chosen as a type or standard strain by the Nomenclature Committee, germinate in typical *equatorial* fashion as first described by Prazmowski 1880, later by Brefeld 1881, and others. The Novy strain as described by Soule[1] is shown to germinate by *stretching* with a complete or partially complete rupture of the spore wall at the equator. Where the rupture is complete the spore wall remains as two caps covering either end of the spore. The spores of a typical strain of *Bacillus cereus* is shown to germinate by extrusion of the vegetative rods at one end of the spore (*polar*) germination. Later the spore coats slip off the ends of the rods and lie free among the vegetative cells.

A further meeting of the Nomenclature Committee took place on Tuesday, July 28th in the Congress Committee Room at 4 p.m. It was mainly concerned with drafting resolutions to be presented for ratification at the Plenary Meeting of the Congress on Saturday, August 1st, 1936.

The following members were present:—Prof. R. S. Breed, Chairman (U.S.A.); Prof. R. E. Buchanan (U.S.A.), Dr. M. Grimes (Irish Free State), Prof. A. J. Kluyver (Holland), Prof. F. Mesnil (France), Prof. S. Orla-Jensen (Denmark), Dr. J. C. N. Penido (Brazil), Dr. A. R. Prévot (France), Prof. E. A. Pribram (U.S.A.), Mr. J. Ramsbottom (Great Britain), Dr. R. St. John-Brooks (Great Britain), Dr. W. M. Scott (Great Britain), Dr. A. C. Thaysen (Great Britain), Mr. P. Bruce White (Great Britain), and Dr. P. Arne Hansen (Denmark) and Dr. H. Schütze (Great Britain) members of the Congress.

Dr. Breed presented the report by himself and Dr. St. John-Brooks on "Proposals Submitted by R. E. Buchanan and H. J. Conn relative to the Conservation of *Bacillus* as a Bacterial Generic Name, Fixing of the Type Species and of the Type or Standard Culture," which had been published in *Zbl. Bakt.* Abt. 2, 1935, **92**, 481. The report was approved by the Committee and the recommendations embodied therein were sent forward for ratification at the Plenary Meeting of the Congress.

Dr. St. John-Brooks presented the Report of the *Salmonella* Subcommittee, which had been published in the *Journal of Hygiene*,

[1] *J. Infect. Dis.*, 1932, **191**, 51.

1934, 34, 333. This embodied the views of the Subcommittee on the subject, but did not necessarily represent the opinions of the members of the Nomenclature Committee itself. Dr. St.John-Brooks mentioned that under Article 4, section *b* of the resolutions of the Nomenclature Committee, authorized and constituted by resolution in plenary session at the Paris Congress, authority and encouragement were given for the monographing of special groups or genera of bacteria. In furtherance of this object a Subcommittee was called together early in 1933 to consider the question of the taxonomy and nomenclature of the *Salmonella* group, Dr. H. Schütze, Lister Institute, London, being appointed Chairman; the other members of the Subcommittee comprising: Prof. E. O. Jordan, University of Chicago, U.S.A., Dr. F. Kauffmann, Statens Seruminstitut, Copenhagen, Denmark; Dr. W. M. Scott, Ministry of Health, London; Mr. P. Bruce White, National Institute for Medical Research, London: with Dr. R. St.John-Brooks, National Collection of Type Cultures (Medical Research Council), Lister Institute, London, as Secretary. The late Dr. J. Lignières, some time before his death consented to serve on the Subcommittee. As, however, he died before he had an opportunity to consider the draft report, which he did not take part in drawing up, his name did not appear as a member of the Subcommittee. Subsequent to the publication of the Report, Prof. Jordan[1] resigned from the Subcommittee.

This report was considered by the members of the Committee and after some discussion the Subcommittee were thanked for their services and, at their request, the report was referred back to them for further consideration and amplification. They were asked to continue their researches, with power to co-opt additional members if they so desired. The Committee expressed the wish that workers in this field should consult the Subcommittee before publishing specific names for newly identified species of *Salmonella*. The Committee as a whole approved the definition of the genus *Salmonella* Lignières as set out in the report and accepted the type species as *Salmonella cholerae-suis* (Smith).

Prof. Mesnil (Paris) presented a proposal framed to avoid the duplication of generic terms among *Protista*, e.g. *Babesia*. The proposal was approved and was sent forward for ratification at the Plenary Meeting of the Congress.

Prof. Mesnil was requested by the Committee to constitute a Subcommittee to examine the existing names of different groups of *Protista* from this point of view, to act as Chairman of this Subcommittee and to present a report at the next Congress.

Prof. Breed (U.S.A.) presented a proposal relative to the non-capitalization of specific names. This proposal was similarly approved by the Committee.

Prof. J. Howard Brown was appointed to draw up a statement for presentation to the Committee regarding the use of abbreviations

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[1] *Obit.* Sept. 2, 1936.

for generic names and was given power to act in appointing a Sub-Committee to work with him.

As a result of these deliberations it was resolved to put forward to the Members of Congress at the Plenary Meeting on Saturday, August 1, 1936, certain Resolutions for general approval. These Resolutions, which are set out in full on pages 28 and 29 of these Proceedings, were subsequently passed by the Plenary Congress.

Appendix

Mr. P. Bruce White (London): *Remarks on the Nomenclature and Classification of Bacteria.*

It is desirable that those who interest themselves in the nomenclature and classification of bacteria should clearly visualize the end to which they strive and should formulate a plan of action.

I venture to place certain considerations and suggestions before my colleagues.

1. Though the authority of the Committee presumably extends only to points of nomenclature, its actual problem lies in collecting that expert opinion regarding the classification, identities and differences of organisms on which alone adjudication of titles can be based. It cannot escape from the evaluation of relationships and the construction of a taxonomic system.

2. In the construction of this system two opposing principles and methods present themselves.

(*a*) One aims at a partition of the bacterial world on those differential criteria which offer the sharpest definition and readiest recognition of any named group, species or variety. Such a partition, illustrated in the so-called "determinative systems," must, almost of necessity, begin with the larger cleavages and thence work downwards by repeated subdivision to the smaller. The basis of such a system may be laid by a group of instructed men with the gift of ingenuity in a few months or years but its liability to grievous error is proportional to its facility and the ambition of its major subdivisions.

(*b*) The second type of system would be inspired by belief in the reality of the relationships and differences suggested, would eschew so far as possible the similarities of convergent evolution and would value superficial divergence, even if morphological and however striking, as trivial. Such a system must develop from multiple foci of intensive study, gradually widening until they become confluent and an opinion can be formed as to the rational groupings of larger size. Such a system must be built slowly but it would be laid on sure foundations; its errors would be in detail only.

3. While we may wish to see a comprehensive classification and nomenclature of bacteria established in our time and to do fitting honour to the labours of the past, we must realize that sooner or later rational taxonomy must be begun and that in the reconstruction

little place may be found for the terminology and conceptions of the pioneers. Let us beware lest by immature decisions and arbitrary definitions we hinder progress. Our precipitation to deal with such crumbling genera as *Bacillus* and *Bacterium* is a case in point.

4. It will be agreed that the earliest possible escape must be made from dependence on biochemical properties in judging all save the most trivial points of relationship and difference. But it must also be recognized that only limited reliance is to be placed on morphological criteria such as the presence or absence of flagella, spores and capsule: non-flagellate and non-sporing species may be but minor divergents of motile and sporing forms.

At the present time, only one method of attack offers promise of real success over a wide field, namely the assembly and partition of bacteria according to the diversities and similarities of their living substance as disclosed by chemical and serological study—not merely on the findings of perfunctory agglutination tests but by methodical analysis of the type—(or species)—specific, genus specific and group specific constituents of the normal organism and its variants. This " substantive " method offers the bulk of the information we require for the competent classification of bacteria. When such information has been collected it will be time to consider subsidiary criteria and the niceties of nomenclature.

5. There are certain types of differential criteria, widely adopted in the past, which must be viewed with suspicion: the separation of organisms on a plus-minus basis and the grouping of others on purely negative characters. So far as it is possible all differentiation should be made on opposing positive qualities and all approximation on positive agreement.

6. The level and nature of the bacterial genus and species is a matter calling for consideration.

It is reasonably clear that such an accepted genus as *Salmonella*, presenting types honoured with specific names, is actually of the same order as pneumococcus, treated usually as a single species enclosing numbered subtypes or varieties. Convenience, history, and the characters of the organisms themselves may favour this difference of treatment but the fact remains that the *Salmonella* type and the pneumococcus type are of equivalent natural status and are elementary species in the sense of de Vries, and that *Salmonella* and *Pneumococcus* are natural genera roughly defined by their R variants and by their basal proteins and polysaccharides.

The Committee might usefully reach a decision as to whether the taxonomic species should be established at the level of the elementary species or whether its size in different cases should continue to be dictated by convenience.

In conclusion I venture to suggest that, in place of endeavouring to salve certain time-honoured relics from the débris of obsolete terminology, we should do all in our power to stimulate those researches which alone can furnish the material for our decisions.

INFORMAL MEETING UNDER THE AUSPICES OF THE HEALTH COMMITTEE OF THE LEAGUE OF NATIONS

Discussion on the Serum Diagnosis of Typhoid Fever

During the course of the Congress a meeting was held to discuss the results of the "International Experiment on the Widal reaction" which had been carried out late in 1935 at the instigation of Prof. Th. Madsen, acting for the Health Committee of the League of Nations. There were present at the meeting: Prof. Th. Madsen (in the chair), Dr. A. Felix, Dr. A. D. Gardner, Dr. F. Kauffmann, Dr. W. M. Scott and Professor W. W. C. Topley.

The aim of the "international experiment" was to ascertain whether greater uniformity could be achieved by different workers using standardized killed suspensions by a uniform technique, than with the diverse suspensions and methods generally employed in the different laboratories.

At Prof. Madsen's suggestion, a meeting of English bacteriologists especially interested in agglutination work, was held at the Lister Institute in London on July 26, 1935. Those present were: Dr. A. Felix (Lister Inst.); Dr. A. D. Gardner (Oxford; Med. Research Counc.); Dr. H. Schütze (Lister Inst.); Dr. W. M. Scott (Ministry of Health); Mr. P. Bruce White (Nat. Inst. Med. Res.). Dr. W. W. C. Topley (Lond. School Hyg. & Trop. Med.) also was consulted. It was agreed that the "standardized suspensions" made at Oxford on behalf of the Medical Research Council were suitable for use in the proposed experiment. A special "standard" technique was recommended for the purposes of the experiment and the details of the technique were agreed upon unanimously.

The international experiment consisted of the titration by each of the participating laboratories of a fraction of each of a large number of sera from human enteric fever, the titration being carried out with

(*a*) Suspensions and technique used for routine in each laboratory.

(*b*) "Standardized suspensions" of *Bact. typhosum*, H and O types, prepared at the Standards Laboratory, Oxford, with the special prearranged "standard" technique.

The following laboratories participated in the experiment:—

Bucarest.—Inst. des Sérums et Vaccins. (Dr. M. Ciuca).

Copenhagen.—Statens Seruminstitut. (Prof. Th. Madsen with Dr. F. Kauffmann).

Oxford.—Medical Research Council's Standards Laboratory, School of Pathology. (Dr. A. D. Gardner).

Warsaw.—L'Inst. d'Hygiène de l'État. (Prof. L. Hirszfeld).

The protocols of all laboratories had been submitted to Dr. A. D. Gardner, who had made a thorough analysis of the figures and

embodied it in a Report, which was the main matter for consideration by the meeting.

This Report was recommended for early publication.[1] Its main conclusion was that greater uniformity and accuracy could be achieved by the use of standardized suspensions and technique.

The meeting further agreed that Dr. Félix and Dr. Gardner should prepare a statement of results of the "international experiment" and recommendations for a standard method, to be sent to Prof. Madsen for circularization to European and other laboratories.

CINEMATOGRAPH DEMONSTRATIONS

The following cinematograph films were shown at The London School of Hygiene and Tropical Medicine, Keppel Street, W.C.1, on Thursday, 30th July, at 5 p.m., and on Friday, 31st July, at 2.30 p.m., under the direction of Dr. J. O. W. Bland.

1. *Germination of Spores of Bacteria*, by Prof. S. Bayne-Jones (Yale University, U.S.A.). Demonstrated by Prof. R. S. Breed (Geneva, U.S.A.).

A film of microphotographs of living cells showing the germination of bacterial spores, was presented.

2. *Management of Diphtheria and Croup*, by Dr. J. G. M. Bullowa (New York).

The diagnosis and treatment of the various manifestations of diphtheria, with the treatment of croup by suction, intubation, tracheotomy and a steam cubicle, were shown. The technique of aseptic care by nurses and medical staff and the method of preventing diphtheria by the injection of alum toxoid were also displayed. The great contributors to knowledge of diphtheria were shown and the method of manufacturing alum toxoid was covered.

3. *Management of Pneumonias*, by Dr. J. G. M. Bullowa (New York).

This film gave the management of the pneumonias at Harlem Hospital. It showed in detail the method of typing sputum, lung suction, administration of serum, and the control of dosage by agglutination reactions. It gave statistics in Types I, II, and VII, as well as the method of treating anoxæmia by catheter, tent and chamber, and the treatment of pulmonary œdema.

4. *Psittacosis Virus. A Study in Tissue Culture*, by the late Dr. R. G. Canti and by Dr. J. O. W. Bland (London).

A study of the growth and development of psittacosis virus in the cells of infected cultures of chick embryo tissues. The mode of formation of the intracellular colonies of the virus and the changes taking place in them in the living cell were shown both by dark ground and ordinary illumination.

5. *Investigations on Immunity in Cancer*, by the late Dr. R. G. Canti and by Dr. T. Lumsden (London).

[1] Gardner, A. D., *J. Hyg.* 1937, 37, 124.

The first two experiments filmed showed the lethal effect of the serum of rats immunized against Jensen's Rat Sarcoma (J.R.S.) upon tissue cultures of J.R.S. These cultures are first re-fed with serum drawn from a normal rat. This has no damaging effect. The immune rat serum is then run on and the J.R.S. cells are rapidly killed.

The later films showed that when normal rat tissues are cultured in the same drop of normal rat serum as J.R.S. cells, an application of J.R.S. immune rat serum leaves the normal cells undamaged ; the malignant cells are killed just as in the first two experiments.

The immune sera were thus shown to have a specific affinity for cancer cells.

DEMONSTRATIONS GIVEN BY MEMBERS OF THE CONGRESS.

Monday, 27th, to Thursday, 30th July, 1936.

The following demonstrations were given by members of the Congress in the Department of Physics, University College, London, by kind permission of Prof. E. N. da C. Andrade.

Dr. Louis Dienes (Boston, U.S.A.). Photomicrographs illustrating the Subjects of his Communication (*see p.* 50).

Dr. J. Drbohlav (Prague). A Portable Incubator for Transmission by Post.

Drs. G. H. Eagles, M. H. Salaman and C. R. Amies (London). Elementary Bodies of Certain Virus Diseases.

Prof. Alexander Fleming (London). The Growth of Microorganisms on Paper (*see Appendix I*).

Dr. Earl W. Flosdorf and Dr. Stuart Mudd (Philadelphia). Demonstration of the Procedure and Apparatus for preservation in " Lyophile " Form of Serum and other Biological Substances.

Prof. Ivan C. Hall (Colorado, U.S.A.). Metabolic " Mutation " in the Genus *Bacterium*.

Lt.-Col. S. P. James (London). The life-cycle of *Plasmodium ovale* in man and mosquito.

Prof. N. von Jancsó (Szeged, Hungary). Methoden zum qualitativen und quantitativen Nachweis chemotherapeutischer Mittel im Körper der Krankheitserreger.

Dr. E. Klieneberger (London). A New Group of Microbes Occurring as Symbionts of Bacteria and Independently.

Prof. R. D. Manwell (Syracuse, U.S.A.). Seven Species of Malaria Parasites with Elongate Gametocytes.

Dr. S. Marbais (Livry-Gargan, France). Apparatus of Use in Clinical Diagnosis.

Prof. E. Reichenow (Hamburg). Development of *Babesia canis* in the tick *Dermacentor reticulatus*.

Dr. S. L. SCHOUTEN (Utrecht, Holland). Isolation and Dissection with the Schouten Micro-manipulator (*see Appendix 2*).

Lt.-Col. H. E. SHORTT (Madras). (*a*) Development of *Babesia canis* in the tick *Rhipicephalus sanguineus*; (*b*) The structure of Negri bodies; (*c*) Sections of *Phlebotomus argentipes* in relation to the Development and Transmission of *Leishmania donovani*.

Dr. G. SHWARTZMAN (New York). Specimens and Photographs illustrating the Subject of his Communication (*see p.* 411.)

Lt.-Col. J. A. SINTON (Kasauli, India). Three species of malarial parasites isolated from the macaque, *Silenus irus*.

Prof. OSWALD STRENG (Helsinki). Blood Groups and Anthropology.

Dr. REDVERS THOMPSON (Ontario, Canada). A Mechanical Device which Prepares and Inoculates Rolled Tubes.

Prof. E. E. TYZZER (Boston, U.S.A.). The Development of *Histomonas meleagridis*, the Parasite causing "Blackhead" in Turkeys.

APPENDIX 1

Prof. A. Fleming (London): *The Growth of Micro-organisms on Paper.*

If a paper disc is placed on the surface of an agar plate the nutrient material diffuses through the paper sufficiently to maintain the growth of many micro-organisms implanted on the surface of the paper. At any stage growth can be stopped by the introduction of formalin. Finally the paper disc, with the culture on its surface, can be removed, dried and suitably mounted.

The nature of the paper makes a considerable difference to the result obtained. On filter-paper good growth takes place, but the extreme porosity of the paper makes the growth diffuse and, in the case of many moulds, the organism grows through the paper and adheres so firmly to the surface of the culture medium that the paper is torn in attempting to remove it from the surface of the medium. For most purposes a good stiff note-paper is suitable. The colonies remain discrete and the paper disc can be easily removed. If the colony is white a black paper disc can be used, but with chromogenic bacteria white paper is preferable.

The method is especially useful for making specimens for museum and teaching purposes. Single colonies of moulds in all stages of development can be preserved dry for an indefinite period. Permanent specimens illustrating such phenomona as selective bacteriostasis, droplet infection, etc. can easily be prepared.

Dried cultures of chromogenic bacteria have been preserved in this way for two years and the colours have not faded except in cases where the specimens had been exposed to light, especially bright sunlight. The colour of *B. prodigiosum* was especially

sensitive to light and faded very considerably in a few months when exposed to diffuse sunlight on a laboratory wall. In similar conditions there was no perceptible fading of the cultures of staphylococcus, sarcina, or *B. violaceum.*

APPENDIX 2

Dr. S. L. Schouten (Utrecht): *The Micro-manipulator.*

The construction and technique are in the main the same as that of the first micromanipulator, demonstrated in 1899 at Haarlem and published in "Handelingen van het 7e Natuur-en Geneeskundig Congres."*

The microscope stands between two needle-holders on an iron plate. The hand continues to lie quietly beside the needle-holder and can with one finger move the screws, by means of which the needles are shifted in all directions. Very tiny movements (e.g., to dissect bacilli) are possible by pushing against the micrometer screw of the microscope. Fast movements (to pierce tough cell walls or catch infusoria) are also possible.

Micropipettes are used, e.g., for micro-injection of liquids, never for isolation. This is done with glass needles, bent at the end in the form of an eyelet; eyelets of 6μ for bacilli (droplets are so tiny, that it is impossible to overlook other cells!), larger eyelets for yeast-cells, infusoria, etc. Bacilli adherent to the coverglass, e.g., tuberculosis, are taken up with a point of ½μ (mistakes likewise impossible!). Pointed needles of 1μ and micro-knives of glass are used in dissection. Tissues are fastened with a micro-pincette. Before they are used the needles are sterilized by putting the end in sulphuric acid and afterwards in ammonia; the same needles can be used for years.

The lateral walls of the isolation-chamber are provided with a slit, which is closed by a viscid fluid through which the needles pass; evaporation even of the smallest drops is impossible.

At the Congress the isolation of micro-organisms and the dissection of bacilli was demonstrated; the simplicity of the technique was evident from the rapidity of the experiment (a few seconds only).

EXHIBITION OF SCIENTIFIC INSTRUMENTS AND APPARATUS

An exhibition of scientific instruments and apparatus, which was organized by the Exhibitions Committee, was held on July 27 to July 29 in the Department of Physics, University College, London, by kind permission of Professor E. N. da C. Andrade.

At this exhibition most of the leading British and Foreign manufacturers having agencies in London were represented.

* See Der Mikromanipulator, *Z. wiss. Mikr. und. Mikr. Technik,* 51, 421 and *Arch. f. exp. Zellforschung,* 17, 429.

SCIENTIFIC VISITS

The following scientific visits were arranged by the Sectional Committees of the Congress :

July 27

Royal Army Medical College, Millbank, London, S.W. Tea was kindly provided by the College authorities.

West Middlesex Drainage Scheme, Mogden. The sewage disposal works of the Middlesex County Council. Visitors were privileged to see the works before the official opening ceremony had taken place.

July 28

Rothamsted Experimental Station, Harpenden, Herts. Afternoon tea was kindly provided by the authorities.

The Research Laboratories of Messrs. J. Lyons & Co., Ltd. Transport and tea were kindly provided by Messrs. J. Lyons & Co.

Research Department of the Distillers' Laboratories, Epsom, Surrey. The Distillers' Company kindly provided tea.

July 29

Laboratories of the Metropolitan Water Board, Nottingham Place, London, W.

Lister Institute, Elstree, Herts. Serum Department, Vaccine-lymph Department and experimental farm of the Lister Institute of Preventive Medicine, London. The visit was organized by Dr. G. F. Petrie, Bacteriologist in charge, and tea was kindly provided by the authorities.

The Royal Botanical Gardens, Kew, Surrey and *The Imperial Mycological Institute, Kew, Surrey.* A special visit was paid to the Herbarium at the Botanical Gardens.

July 30

Research Laboratories of United Dairies, Ltd., Shepherd's Bush, London, W. Transport and tea were kindly provided by United Dairies, Ltd.

Metropolitan Water Board, Kempton Park, Middlesex. Reservoirs and water control of the Metropolitan Water Board. The tour extended from the natural water intake to the domestic water output.

July 31

Research Laboratories, General Electric Company, Ltd., Wembley, Middlesex. The General Electric Company kindly provided transport and tea.

Wellcome Physiological Research Laboratories, Langley Court, Beckenham, Kent. The visit was organized by the Director, Dr. R. A. O'Brien, and tea was kindly provided by the Laboratories.

National Institute for Research in Dairying, Shinfield, near Reading, Berks. Transport between Reading Station and the Institute and tea were kindly provided by the Institute.

The Agricultural Research Station of Imperial Chemical Industries, Jealott's Hill, Berks. Imperial Chemical Industries kindly provided tea.

AUGUST 1

Forest Products Research Laboratory, Princes Risborough, Bucks. A day excursion was made to the Research Station.

The following list of institutions in and around London where microbiology is studied and practised was published in the Final Programme and Handbook of the Congress. All these centres were open to receive informal visits from members of the Congress and many availed themselves of the opportunities presented. In a number of cases special arrangements were made for the instruction and entertainment of visitors :

British Leather Manufacturers' Research Association Laboratories, 18-20, St. Thomas Street, London, S.E.

British Postgraduate Medical School (Univ. of London), Ducane Road, Hammersmith, London, W.

Hospital for Sick Children, Great Ormond Street, London, W.C.

East Malling Research Station, East Malling, Kent.

Forest Products Research Laboratory, Princes Risborough, Bucks.

Government Lymph Establishment, Colindale Avenue, Hendon, London, N.W.

Guy's Hospital Medical School (Univ. of London), St. Thomas's Street, London, S.E.

Imperial Bureau of Agricultural Parasitology and Institute of Agricultural Parasitology, Winches Farm Drive, Hatfield Road, St. Albans.

Hospital for Tropical Diseases, 25, Gordon Street, W.C.

Imperial Bureau of Animal Health, New Haw, Weybridge, Surrey.

Imperial Bureau of Soil Science, Harpenden, Herts.

Imperial Bureau of Plant Genetics, School of Agriculture, Downing Street, Cambridge.

Imperial Cancer Research Fund, 8, Queen's Square, London, W.C., and Stroud Farm, The Ridgeway, Mill Hill, London, N.W.

Imperial College of Science and Technology, Prince Consort Road, South Kensington, London, S.W.

Imperial Mycological Institute, Ferry Lane, Kew, Surrey.

John Innes Horticultural Institute, Merton Park, 21, Mostyn Road, London, S.W.

King's College Hospital (Univ. of London), Denmark Hill, London, S.E.

Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.

London County Council, Archway Group Laboratory, Archway Hospital, Highgate, London, N.

London County Council, Belmont Laboratories, Stanley Road, Sutton, Surrey.

London County Council, Central Histological Laboratory, Archway Hospital, Highgate, London, N.

London County Council, Group Laboratory, North-Western Hospital, 4-8, Pond Street, Hampstead, London, N.W.

London County Council, Lambeth Group Laboratory, Lambeth Hospital, Brook Street, London, S.E.

London County Council, Lewisham Hospital, Lewisham, London, S.E.

London County Council, Mile End Group Laboratory, Mile End Hospital, Bancroft Road, Mile End, London, E.

London County Council, St. Abbots Group Laboratory, St. Mary Abbotts Hospital, Marloes Road, Kensington, London, W.

London County Council, Southern Group Laboratory, Park Hospital, London, S.E.

The London Hospital Medical College (Univ. of London), Whitechapel, London, E.

London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.

Middlesex Hospital Medical School (Univ. of London), Mortimer Street, London, W.

National Institute for Research in Dairying (Univ. of Reading), Shinfield, near Reading, Berks.

Ministry of Agriculture and Fisheries, Veterinary Laboratory, New Haw, Weybridge, Surrey.

Ministry of Health, Malaria Laboratory, Horton Mental Hospital, Epsom, Surrey.

Ministry of Health Pathological Laboratory, Dudley House, Endell Street, London, W.C.

National Collection of Type Cultures (Medical Research Council), Lister Institute, Chelsea Bridge Road, London, S.W.

National Institute for Medical Research (Medical Research Council), Mount Vernon, Hampstead, N.W.

Research Institute in Animal Pathology, Great College Street, Camden Town, London, N.W.

Rothamsted Experimental Station, Harpenden, Herts.

The Cancer Hospital (Free) (Univ. of London), Fulham Road, London, S.W.

Royal Army Medical College, Millbank, London, S.W.

St. Bartholomew's Hospital (Univ. of London), Smithfield, London, E.C.

St. Thomas's Hospital (Univ. of London), Lambeth Road, London, S.E.

University College, London (Univ. of London), Gower Street, London, W.C.

University College Hospital Medical School (Univ. of London), University Street, London, W.C.

The Wellcome Research Institution, 183-193, Euston Road, and the Wellcome Entomological Field Laboratories, Claremont, Esher, Surrey.

Westminster Hospital (Univ. of London), Broad Sanctuary, London, S.W.

GENERAL CONGRESS EXCURSIONS

The following general excursions were arranged by the Ladies' Committee and were open to all Members as well as to Members' guests :

July 26

Sightseeing Drive in Buckinghamshire, accompanied by guide-lecturer. Among places visited were Chalfont (Milton's Cottage), Jordans (Penn Burial Ground) and Beaconsfield.

Sightseeing Drive to Windsor and Eton, accompanied by guide-lecturer. The Lower Wards of Windsor Castle were visited.

July 27

National Gallery of Art, London. Guide-lecturers were in attendance.

Drive to Surrey Hills. Guildford, The Hog's Back, The Devil's Punch Bowl and Farnham were visited. A guide-lecturer accompanied the excursion.

July 28

Visit to the Bakeries and Factories of Messrs. J. Lyons & Co., Ltd. Transport was kindly provided by Messrs. J. Lyons & Co.

Visit to Hampton Court, accompanied by guide-lecturer.

July 29

Tour of the West End of London, including visits to the Houses of Parliament and Westminster Abbey. Several Members of Parliament kindly consented to show visitors over the House of Commons.

A visit to the Open Air Theatre, Regent's Park, London, was arranged for the afternoon. A performance of Shakespeare's " A Midsummer Night's Dream " was witnessed.

July 30

Visit to the Tate Gallery, Millbank, London, S.W. Guide-lecturers were in attendance.

JULY 31

Tour of the City of London, including the Tower of London and the Guildhall. A guide-lecturer accompanied the excursion.

Visit to Imber Court, Surrey. The centre for training police horses.

Dress Show and Mannequin Parade at Messrs. Liberty & Co., Regent Street, London, W. Tea was kindly provided by Messrs. Liberty & Co. Guide-interpreters were in attendance.

AUGUST 1

Excursion to Oxford University. A day excursion was made to the University, which included visits to some of the Colleges and to the Department of Pathology. The visit was arranged by Dr. A. D. Gardner, Dept. of Pathology, Oxford University.

AUGUST 2

Excursion to Cambridge University. The Master and Fellows of Emmanuel College kindly entertained the visitors to lunch and the Master of Trinity Hall kindly entertained them to tea. Laboratories in the Departments of Pathology and Parasitology were open for inspection and demonstrations were given. The visit was arranged by Prof H. R. Dean and Dr. A. N. Drury, Dept. of Pathology, Cambridge University.

ZOOLOGICAL SOCIETY OF LONDON.

Tickets for admission to the Zoological Gardens, Regent's Park, London, N.W., or to Whipsnade Park, near Dunstable, Bedfordshire, were kindly presented by Dr. Julian S. Huxley, Secretary of the Zoological Society of London. A large number of members availed themselves of this privilege.

A special performance of the cinematograph film "The Story of Louis Pasteur" was given on July 30 at the New Gallery, Regent Street, London to the members of the Congress, through the courtesy of the Management.

OFFICIAL GOVERNMENT DELEGATES, OF WHICH THOSE IN ATTENDANCE WERE PRESENTED TO THE LORD PRESIDENT OF THE COUNCIL AT THE OPENING CEREMONY ON SATURDAY, JULY 25TH, 1936.

ANGLO-EGYPTIAN SUDAN
Dr. E. S. Horgan

BELGIUM
Prof. J. Bordet Prof. E. Renaux
Prof. R. Bruynoghe

BULGARIA
Prof. St. Angeloff

CANADA, DOMINION OF
Dr. W. H. Cook

CEYLON
Mr. M. Park

CHINA
Prof. R. H. P. Sia Dr. Fei Fang Tang
Dr. Shan Min Tao

CYPRUS
Dr. R. M. Nattrass

CZECHOSLOVAKIA
Dr. J. J. Drbohlav Dr. C. Hruška
Dr. Ludmilla Mlčochová

DENMARK
Prof. O. Thomsen

DUTCH EAST INDIES
Dr. J. W. Wolff

EGYPT
Prof. Mahmoud Abdul Rehim Selim Dr. Aly Hassan
Dr. Hassan Helmy Salem
Dr. Abdel Hamid Gohar

ESTONIA

Prof. K. Schlossmann

FEDERATED MALAY STATES AND STRAITS SETTLEMENTS

Dr. H. M. Nevin
Mr. R. A. Altson
Mr. G. Rocker

FIJI

Mr. H. W. Simmonds

FINLAND

Prof. O. Streng

FRANCE

Prof. E. Brumpt
Prof. G. Lavier

GERMANY

Prof. R. Otto (Head of Delegation)
Prof. W. Schwartz
Prof. K. Herzberg
Prof. P. Uhlenhuth
Prof. R Prigge
Dr. H Habs
Prof. E Gotschlich
Prof. E. Rodenwaldt
Prof. E. Reichenow
Dr. H. Glathe
Prof. K. Laubenheimer
Dr. K. J. Demeter
Prof. O. Meyerhof
Prof. H. Schmidt
Prof. H. Miessner
Dr. W. Lehmann
Prof. K. Jötten
Prof. F. Sartorius
Prof. R Bieling
Dr. H Gaffron
Prof. K. Poppe
Dr. Schäffer
Dr. A. v. Mallinckrodt-Haupt
Dr. J. Zeissler

GOLD COAST

Dr. G. F. T. Saunders

GREAT BRITAIN AND NORTHERN IRELAND

Dr. E. Mellanby (Chief Delegate)
Mr. T. Anderson
Dr. W. Horner Andrews
Dr. J. L. Brownlie
Dr. E. J. Butler
Dr. R. W. Dodgson
Mr. H. G. Lamont
Sir Arthur S. MacNalty
Dr. G. H. Pethybridge
Mr. J. Ramsbottom
Sir Frank E. Smith

GREECE

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Dr. G. Stefanopoulo

HOLLAND

Prof. A. J. Kluyver (Chief Delegate)
Prof. W. A. P. Schüffner
Prof. Johanna Westerdijk
Prof. P. C. *Flu*
Prof. J. J. van Loghem

HONG KONG

Major W. J. E. Mackenzie

HUNGARY

Prof. J. von Daranyi
Dr. B. Johan

INDIA

Mr. J. R. Haddow

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M. Suleyman Sadigh Ershadi

KENYA

Mr. W. B. C. Danks

MALTA

Dr. V. J. Mifsud

NEW SOUTH WALES

Dr. E. S. Morris

NIGERIA

Dr. E. C. Smith

PALESTINE

Dr. G. Stuart

POLAND

Prof. Th. Chrzaszcz
Prof. F. von Gröer
Prof. St. Legeżyński
Dr. J. Celarek

QUEENSLAND

Dr. J. V. Duhig

ROUMANIA

Dr. I. Bazgan

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SOUTH AFRICA, UNION OF
Sir Arnold Theiler

SOUTHERN RHODESIA
Dr. D. O. Richards*

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Dr. A. R. Falcó   Dr. J. J. Fernandez de la Reguera

SWEDEN
Prof. J. Forssman

TANGANYIKA TERRITORY
Mr. H. E. Hornby   Mr. H. B. Stent

UGANDA
Dr. Mary Turton   Mr. G. F. Clay
Mr. J. Carmichael

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Dr. Sara E. Branham
Prof. R. E. Buchanan
Prof. E. G. Conklin
Dr. Alice C. Evans
Prof. K. Landsteiner
Dr. K. S. Markley
Dr. E. B. McKinley
Prof. W. H. Park
Dr. T. M. Rivers
Prof. E. W. Schultz
Dr. W. Trelease
Prof. H. Zinsser

NOTE: The interests of the Government of Jamaica at the Congress were represented by Mr. S. F. Ashby, Director of the Imperial Mycological Institute.

MEMBERS OF THE SECOND INTERNATIONAL CONGRESS FOR MICROBIOLOGY, LONDON, 1936

Algeria
Fabiani, Dr. G.
Pinoy, Prof. E.
Sergent, Prof. E.

Anglo-Egyptian Sudan
Archibald, Sir Robert
Horgan, Dr. E. S.
Massey, Mr. R. E.

Annam
Yersin, Prof. A.

Argentina
Ponce de Leon, Prof. R. S.
Sordelli, Prof. A.

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Mackerras, Dr. M. J.
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Reid, Dr. C. A.
Resch, Dr. C. E.
Turner, Dr. A. W.
Young, Prof. W. J.[2]

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Csonka, Dr. J.
Diernhofer, Prof. K.
Engling, Prof. M.
Gerlach, Dr. F.
Grassberger, Prof. R.
Jettmar, Dr. H.
Loewenstein, Prof. E.
Silberstein, Prof. F.
Weiss, Dr. T.

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Bruynoghe, Prof. R.
Dupont, Mlle. M.
Le Fèvre de Arric, Dr. M.
Gratia, Prof. A.
Jadin, Dr. J.
Linz, Dr. R.
Millet, Dr. M.
Muthaars, Dr. W.
Renaux, Dr. Emile
Renaux, Prof. Ernest
Rodhain, Prof. J.
Ronse, Mlle. M.
Steyaert, Dr. R. L.

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Da Fonseca, Dr. O.
Fontes, Prof. A.
Magalhães, Dr. O.
Moses, Dr. A.
Penido, Dr. J. C. N.
Regendanz, Dr. P.

Bulgaria
Angeloff, Prof. St.
Angeloff, Mme. St.

Canada, Dominion of
Campbell, Dr. J. F.
Cook, Dr. W. H.
Cosgrave, Miss M.
Craigie, Dr. J.
Dolman, Prof. C. E.
Fitzgerald, Prof. J. G.
Lucas, Prof. C. C.
McKinnon, Dr. N. E.
Murray, Prof. E. G. D.
Pett, Dr. L. Bradley
Silverthorn, Dr. N.
Smith, Dr. F.
Thompson, Dr. R.
Vanterpool, Prof. T. C.
White, Dr. F. D.
Wyllie, Prof. J.

Ceylon
Park, Mr. M.

Channel Isles
Wilson, Dr. R. Arderne

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Read, Prof. B. E.
Sia, Prof. R. H. P.[3]
Tang, Dr. F. F.[3]
Tao, Dr. S. M.[4]
Wang, Miss F. R.
Yen, Dr. A. C. H.

Cuba
Hoffmann, Prof. W. H.

Cyprus[*]
Nattrass, Dr. R. M.

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[1] Representative of the University of Queensland.
[2] Delegate of the University of Melbourne.
[3] Delegate of the Chinese Society of Pathology and Microbiology, Shanghai.
[4] Representative of the National Health Administration, Nanking.

Czechoslovakia
Czurda, Prof. V.
Drbohlav, Dr. J. J.[5]
Drbohlav, Mrs.[5]
Felsenfeld, Dr. O.
Fingerland, Dr. A.
Haurowitz, Prof. F.
Hruška, Dr. C.
Jírovec, Dr. O.
Mlčochova, Dr. L.
Patočka, Dr. F.[6]
Singer, Prof. E.

Denmark
Bang, Prof. O.
Bovien, Dr. P. L.
Engelbreth-Holm, Dr. J.
Fjord-Nielsen, Dr. I.
Friedenreich, Dr. V.
Hansen, Dr. P. Arne
Jensen, Dr. Vilh.
Kauffmann, Dr. F.
Kristensen, Dr. M.
Madsen, Prof. Th.
Nielsen, Dr. N.
Orla-Jensen, Prof. S.
Ørskov, Prof. J.
Thomsen, Prof. O.

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Wolff, Dr. J. W.

Egypt
Abdush, Dr. Y. B.
Compton, Dr. A.
El Alaily, Dr. Y. A.
El Seifi, Dr. M. F.
Forsyth, Prof. W. L.[7]
Gohar, Dr. A. H.[7]
Hassan, Dr. A.[7]
Matta, Dr. D.
Panayotatou, Dr. A.
Salem, Dr. H. H.[7]
Selim, Prof. M. A.

Estonia
Leisman, Dr. L.
Schlossmann, Prof. K.

Federated Malay States and Straits Settlements
Altson, Mr. R. A.
Nevin, Dr. H. M.
Rocker, Mr. G.
Young, Prof. W. A.

Fiji
Simmonds, Mr. H. W.

Finland
Karström, Dr. H.
Packalen, Dr. T.
Streng, Prof. O.
Virtanen, Prof. A. I.

France
Aïtoff, Dr. M.
Belin, Dr. M.
Berthelot, Dr. A.
Boivin, Prof. A.
Bovet, Dr. D.
Brumpt, Prof. E.
Butavand, Dr. A.
Cailleau, Mlle. R.
Cathoire, Dr. L.
Chaix, Dr. P.
Cotoni, Dr. L.
Deschiens, Dr. R. E.
Dienert, Dr. F.
Dieryck, Dr. J.
Dujardin-Beaumetz, Dr. E.
Dujarric de la Rivière, Dr. R.
Durel, Dr. P.
Fernbach, Prof. A.
Fromageot, Prof. C.
Gamaleia, Dr. T.
Giroud, Dr. P.
Goldie, Dr. H.
Guillaumie, Mlle. M.
Hauduroy, Dr. P.
Kossovitch, Dr. N.
Langeron, Dr. M.
Laroux, Dr. P.
Lavier, Prof. G.[8]
Lépine, Dr. P.
Levaditi, Prof. C.
Levy-Bruhl, Dr. M.
Lupu, Dr. A.
Lwoff, Dr. A.
Lwoff, Mme. M.
Macheboeuf, Prof. M.
Maignon, Prof. F.
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